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EXPERIMENTS ON THE VASOCONSTRICTOR ACTION OF BLOOD SERUM *

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AND
EDWARDS A. PARK, M.D.
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PART 1

INTRODUCTION

The power of blood after clotting or defibrination to constrict blood vessels, as evidenced by its action on perfused organs or on the excised arterial strip, has been frequently observed and to some extent studied as to its nature and origin. The work here presented has shaped itself into a study of the vasoconstrictor substance in relation, first, to unclotted blood; second, to the cellular and noncellular elements of the blood; third, to certain biologic, physical and chemical reactions; and lastly, to the process of coagulation. The literature will be reviewed in much the same order. External events have brought the work to a close while it was in many respects incomplete; nevertheless we feel justified in recording it as it stands. It was suggested by the work of two of us[1] in 1912 and certain of the conclusions as to the origin of the vasoconstrictor substance from blood platelets and its ability to resist heat or pass through a celloidin membrane were arrived at independently, although a more thorough search of the literature revealed previous work on the same points.

LITERATURE

The stimulating action of blood serum, or defibrinated blood, on the arterial wall has long been known. As early as 1869 Ludwig and Schmidt[2] on perfusing the muscle of the dog, encountered an unex-

* Submitted for publication March 1, 1918.

* From the Medical and Pediatric Departments of the Hunterian Laboratory, Johns Hopkins University.

* The experiments described in this article were terminated before completion because Drs. Janeway and Park entered on war duties. The paper was written by Dr. Richardson. All of the writing except Part III was supervised by Dr. Janeway before his death.

1. Janeway, T. C., and Park, E. A.: Jour. Exper. Med., 1912, **16**, 541.

2. Ludwig, C., and Schmidt, A.: Arb. a. d. physiol. Anstalt z. Leipzig, 1868, p. 1.

pected resistance to the flow of defibrinated blood. Five years later Mosso,[3] on beginning to perfuse the kidney of the dog, found that the defibrinated blood used failed for several minutes to flow. These observations were confirmed by a number of observers, including Bernstein,[4] Pfaff and Tyrode,[5] Battelli,[6] and others to be mentioned. They used a variety of preparations, including all manner of perfused organs, as well as the excised strip of ox artery developed by Meyer.[7] Two main explanations for the phenomenon were given. Stevens and Lee[8] and Brodie[9] attributed it to some substance formed during coagulation, whereas Meyer,[7] Schlayer,[10] and Bröking and Trendelenburg[11] attributed it to epinephrin.

This question was settled mainly by O'Connor,[12] who first tested the serum by means of a pair of biologic preparations, the first of which reacted to epinephrin by constriction, the second by relaxation. Such a pair was the frog perfusion preparation and the excised segment of rabbit intestine. Serum proved to constrict both, and further, resisted procedures calculated to destroy epinephrin. Moreover, by comparing plasma with the corresponding serum, O'Connor was able to demonstrate that the "substances which simulate epinephrin get into the blood during the process of clotting."* Investigation of plasma from the peripheral circulation by the same method failed to give evidence of epinephrin. Very shortly afterwards Stewart[13] recommended a similar pair of test objects and by the use of them was led to the conclusion that "no evidence is obtained of the presence of adrenalin [epinephrin] in detectable amounts in normal blood taken from the general circulation."

The work of O'Connor is also in accord with the observations mentioned of Stevens and Lee[8] and of Brodie[9] as to the relation between coagulation and the production of vasoconstrictor power. It was con-

3. Mosso, A.: Arb. a. d. physiol. Anstalt z. Leipzig, 1874, **9,** 156.
4. Bernstein, J.: Pflüger's Arch. f. path. Anat., 1877, **15,** 575.
5. Pfaff, F., and Vejnx-Tyrode, M.: Arch. f. exper. Path. u. Pharmakol., 1903, **49,** 324.
6. Batelli, F.: Jour. de physiol. et de path. gén., 1905, **7,** 625, *Idem.* p. 651.
7. Meyer, O. B.: Ztschr. f. Biol., 1906, **48,** 352.
8. Stevens, L. T., and Lee, F. S.: Studies Biol. Lab., Johns Hopkins Univ., 1884, **3,** 99.
9. Brodie, T. G.: Jour. Physiol., 1903, **29,** 266.
10. Schlayer: Deutsch. med, Wchnschr., 1907, **33,** 1897.
11. Broeking, E., and Trendelenberg, P.: Deutsch. Arch. f. klin. Med., 1911, **103,** 168.
12. O'Connor, J. M.: München. med. Wchnschr., 1911, **58,** 1439. Ibid., Arch. f. exper. Path. u. Pharmakol., 1912, **67,** 195.
13. Stewart, G. N.: Jour. Exper. Med., 1911, **14,** 377

* This and following quotations from foreign journals are translations made by H. B. R.

firmed, at least in part, by Trendelenburg.[14] Schultz,[15] in studying another problem, obtained the same result, which has since been confirmed by a number of observers. Stewart,[16] using a properly chosen pair of biologic test objects, failed to find evidence of epinephrin in human serums. Stewart and Harvey[17] confirmed O'Connor's results in regard to the formation of the vasoconstrictor substance during coagulation, and, moreover, demonstrated its power to constrict the coronary as well as the peripheral arteries. Janeway and Park,[1] using parallel preparations of carotid and coronary, were able to "confirm completely the conclusion of O'Connor, Stewart, and Schultz, that the vasoconstrictor substance found in blood serum was not epinephrin." They observed no effect due to uncoagulated blood of ox or rabbit. Further, "the examination of uncoagulated blood from six persons with high blood pressure failed to show the presence of epinephrin or other constricting substances."

The vasoconstrictor substance of serum is therefore not epinephrin. Further proof to this effect is furnished by the experiments in which apocodein prevented the vasoconstrictor action of epinephrin, but not that of serum. For example, Battelli[6] states that apocodein retards, but does not prevent, the action of the "vasoconstrictines" of serum. Stewart and Harvey[17] noted that a dog's kidney, after perfusion with apocodein in sufficient concentration to destroy its sensitiveness to epinephrin, still reacted by diminution of flow to boiled serum. The like was found by Kaufman[18] to be true of the perfused ear of rabbit.

The explanation of the origin of the vasoconstrictor power of serum was more closely approached by Stewart and Zucker[19] in their exhaustive study on serum as compared to plasma and other body fluids on various biologic preparations. Of these the frog perfusion preparation and the excised arterial strip gave little or no reaction to citrated plasma and a marked constrictor action to serum to which citrate had been subsequently added. Between herudin plasma and serum to which herudin had been added there was a difference "not so strongly marked as in the case of the citrate material." They concluded that the vasoconstrictor property is developed during the process of coagulation, and "not mainly" in connection with the actual change of fibrinogen to fibrin, and is associated with changes undergone probably by formed elements of the blood when it is shed.

This last conclusion leads to the relation between the vasocon-

14. Trendelenberg, P.: München. med. Wchnschr., 1911, **58,** 1919.
15. Schultz, W. H.: Bull. 80, Hyg. Lab., U. S. P. H. and M.-H. S., 1912, p. 37.
16. Stewart, G. N.: Jour. Exper. Med., 1912, **15,** 547.
17. Stewart, H. A., and Harvey, S. C.: Jour. Exper. Med., 1912, **16,** 103.
18. Kaufman, P.: Zentralbl. f. Physiol., 1913, **27,** 527.
19. Stewart, G. N., and Zucker, T. F.: Jour. Exper. Med., 1913, **17,** 152; *Ibid.*, 1913, **17,** 174.

strictor action of serum and the formed elements of the blood. On page 172 of the article cited Stewart appends a footnote, "Since this was written we have found that extracts of platelets obtained from citrate blood, the plasma of which is totally inactive, exert a strong constricting effect on artery rings." O'Connor[12] had also mentioned in passing that "extracts of blood platelets of rabbit, obtained from citrated plasma by centrifuging, decanting and dissolving the sediment, rich in platelets, have a powerful effect on the frog vessels." Zucker and Stewart[20] confirmed their previous observation, as did Le Sourd and Pagniez[21] for platelets of a species different from that which furnished the excised artery used for the test.

The few observations on record as to the vasoconstrictor effect of erythrocytes are less in agreement. All these were made on the frog perfusion preparation. Heubner,[22] in studying viscosity, noted in a single experiment that defibrinated blood, if laked and restored to tonicity, causes less constriction of the frog vessels than if diluted with a corresponding amount of Locke's solution. Bröking and Trendelenburg observed that diluted serum caused more vasoconstriction than laked erythrocytes diluted to produce the same viscosity. Hirschfeld and Modrakowski[23] observed, with erythrocytes laked either by means of distilled water or by a specific hemolytic reaction, a marked vasoconstrictor effect. In two experiments, however, the erythrocytes laked by the former method caused dilatation. These observers failed to record the exact speed of centrifuging or to give evidence of any attempt to rid the cells examined of blood platelets, which are in themselves extremely active. Kaufman,[18] who states that "when we laked thoroughly-washed erythrocytes in distilled water or ether, we obtained a strongly vasoconstrictor fluid," is open to a similar criticism.

Studies of relation of the leukocytes to the vasoconstrictor substance have been limited to the effect of extracts of certain organs. Of these, spleen, bone marrow, and thymus may conceivably be regarded as analogous to blood cells. According to Zucker and Stewart[20] these organs, extracted by a method which yielded from the platelets powerful vasoconstrictor substance, are inactive. Kaufman,[24] though agreeing as to the thymus, obtained an active substance from the spleen.

The effect of plasma as compared to serum has been discussed. The effect of plasma as compared to Locke's solution needs further comment. It is to be noted that many observers diluted the plasma before testing it, or else used a considerable percentage of citrate. In

20. Zucker, T. F., and Stewart, G. N.: Zentralbl. f. Physiol., 1913, **27,** 85.
21. Le Sourd, L., and Pagniez, Ph.: Compt. rend. Soc. de biol., 1914, **76,** 587.
22. Heubner, W.: Arch. f. exper. Path. u. Pharmakol., 1905, **53,** 218.
23. Hirschfeld, L., and Modrakowski, G.: München. med. Wchnschr., 1911, **58,** 1494.
24. Kaufman, P.: Zentralbl. f. Physiol., 1913, **27,** 530.

our present experience this substance is deleterious in concentration of 0.5 per cent. within a few minutes. In a previous publication of two of us,[1] as well as that of Sakai and Hiramatsu,[25] the same effect was noted. O'Connor,[12] who stated that plasma had no effect, Stewart and Zucker,[19] that it had little or no effect, and Sakai and Hiramatsu,[25] that it had a vanishingly small effect, diluted the plasma before testing it. Stewart and Zucker used a concentration of citrate as great as 1 per cent. Kaufman,[18] on the other hand, who observed a vasoconstriction, used undiluted citrated plasma. Further, when neither of the objections mentioned apply, as with herudin plasma, more or less vasoconstrictor effect was noted. (Trendelenburg,[14] Kahn,[26] Stewart and Harvey.[17]) Proof is therefore lacking that shed plasma has no tonic effect on the arterial wall; still more obscure is the question whether the circulating plasma has a tonic effect on the intact vessels.

The action of serum on smooth muscle, as opposed to the action of epinephrin, has already been discussed. Attempts to define its chemical nature have yielded contradictory results, even on so elementary a point as its classification as protein or crystalloid. The former alternative is supported by Battelli,[6] who observed that the vasoconstrictor substance of serum was destroyed at a temperature of from 56 to 58 C. by Handovsky and Pick,[27] who observed that it failed to pass through parchment, and that it was not found in the fraction incoagulable by boiling. Kaufman[18] found it insoluble in alcohol or ether. Yanagawa[28] attributes to albumin the action of serum in decreasing the perfusion rate of the excised mammalian heart. Interpretation of his results from the present point of view is complicated by the fact that the hearts used continued to beat. Sakai and Hiramatsu[29] confirmed the failure of the active substance to dialyze through parchment, to resist boiling or to be taken up in alcohol, but unlike Yanagawa, state that it is precipitated with the globulin fraction.

The nonprotein nature of the substance is supported by the work of Schlayer,[10] who observed that it was destroyed neither by a temperature of 56 C., nor by removal of the proteins. His observation that it is not diminished by fifty-eight hours of dialysis is coupled with the statement that epinephrin dialyzes very poorly. This is so at variance with the work of Comesatti[31] that it is permissible to doubt the per-

25. Sakai, S., and Hiramatsu, T.: Mitt. a. d. med. Fakult. d. k. Univ. zu Tokyo, 1914, **13,** 177.

26. Kahn, R. H.: Pflüger's Arch., 1912, **144,** 251.

27. Handovsky, H., and Pick, E. P.: Arch. f. exper. Path. u. Pharmakol., 1912, **71,** 62.

28. Yanagawa, H.: Jour. Pharmacol and Exper. Therap., 1916, **8,** 89.

29. Sakai, S., and Hiramatsu, T.: Mitt. a. d. med. Fakult. d. k. Univ. zu Tokyo, 1916, **15,** 397.

30. Voegtlin, C., and Macht, D. I.: Jour. Am. Med. Assn., 1913, **61,** 2136.

31. Comesatti, G.: Arch. f. exper. Path. u. Pharmakol., 1909, **60,** 233.

meability of the membrane used. Stewart and Harvey[17] state that the vasoconstrictor substance resists boiling and is soluble in alcohol; Stewart and Zucker[19] that it resists boiling and is insoluble in alcohol; Zucker and Stewart[20] that it can be extracted with alcohol, also by acetone and ether. The last-named observer says, moreover, that platelet extract also can be extracted with alcohol. Kaufman[68] found that the active substance, though insoluble in alcohol, resists boiling and dialyzes readily through an artificial membrane, and that the dialyzate gave negative tests for protein. Voegtlin and Macht,[30] by extracting dehydrated blood, serum, plasma, or erythrocytes with chloroform and extracting the dried filtrate with methyl alcohol, obtained a "white crystalline residue" related to cholesterol. This substance resisted boiling in alkaline mediums, and had a tonic action on the vessels of the perfused frog preparation and of the rabbit's ear, as well as on the heart of cold-blooded animals. A substance of the same characteristics was isolated from the adrenal cortex.

If all the above observations hold good, one must conclude that the vasoconstrictor substance is and is not dialyzable, does and does not resist heat, if a protein is or is not a globulin, and if a crystalloid possesses extreme versatility in its ability to be extracted by various solvents. Perhaps a part of the confusion may be explained by absorption of the active substance by the colloids of the blood. In the study of the effects of platelet extract the bulk of these are removed by the simple process of decantation.

That the vasoconstrictor substance is elaborated during the process of coagulation is clear; that the relation is more than a coincidence, is not. Specimens of herudinized blood or plasma which are actively vasoconstrictor do not necessarily contain a clot (Kahn[26]), nor does clotting necessarily occur later. (Kahn,[26] Stewart and Zucker.[19]) The latter[32] found no difference between clotted and unclotted cystic fluid in its effect on the arterial ring, and had previously concluded, as mentioned, that the vasoconstrictor property is developed "not mainly in connection with the actual change of fibrinogen to fibrin."

The foregoing review of the literature reveals a general agreement to the effect that blood serum is vasoconstrictive, and that this action is not due to epinephrin. The rather fragmentary observations on the effect of platelet extract are also in accord, to the effect that the extract has a powerful vasoconstrictor action. The work on erythrocytes is inconclusive; on the leukocytes, of questionable relevancy. The question of the vasoconstrictor effect of circulating plasma is not settled, and the relation of the vasoconstrictor substance to the factors concerned in coagulation has hardly been touched on. The following is an attempt to throw light on these questions.

32. Stewart, G. N., and Zucker, T. F.: Jour. Exper. Med., 1913, **17**, 174.

Technic.—The test object used throughout was the excised strip of ox carotid, as described by O. B. Meyer.[5] Arteries of the dog and the pig were tried, but found to be too insensitive. The apparatus consisted of a large tank suitable for warming the material to be tested, and was kept at body temperature by means of an electric thermostat which gave a very constant temperature within half a degree centigrade. The apparatus was grounded to prevent the accumulation of electrical charges. A small glass tube suitably bent served both as an attachment for the lower end of the artery and as a tube for the ingress of oxygen, a constant flow of which was insured by the use of a large tank. The upper end of the arterial strip was attached to a very light aluminum lever which magnified 15½. The apparatus was so arranged that the artery could be attached at ease to tube and lever, and all three lowered into the arterial chamber. This was made from an ordinary Wassermann tube, and had a capacity of 8 c.c., although 3 or 4 c.c. sufficed to cover the artery. The chamber was suspended in the warm bath. It could be emptied by suction through a suitably bent glass tube fused to the bottom. This arrangement permitted a rapid and complete exchange of fluids with little mechanical disturbance. Such short exposure to air, and even exposure as long as five minutes, did no demonstrable damage to the artery. The apparatus was so arranged that two arterial strips could write simultaneously on the same drum.

The arteries were obtained from a slaughterhouse about one hour's journey from the laboratory. They were taken from the animal within a few minutes of death, and were put in a bottle containing about 800 c.c. of cold Ringer-Locke solution. They were transported and kept on ice. They continued viable for from one to four days, according to the season. Transfer into fresh cold Ringer-Locke seemed to improve their viability.

A segment of artery about 8 mm. long was cut, usually from about the central portion of the artery as obtained. The contracted peripheral end seemed equally sensitive, except in hot weather. The segment was slit longitudinally, so that the tension was exerted on the circular fibers. A needle was passed through the corners at one end of a strip, and a silk ligature tied central to the needle. The ligature was then threaded and drawn through. The same process was repeated at the other end. This procedure prevented slipping of the ligature. The strip, except as otherwise indicated, was stretched by 90 gm. tension for twenty minutes and then weighted with 30 gm. for the tests.

The physiologic fluid used will be referred to as Ringer-Locke solution. It contained:

Sodium chlorid (NaCl), 0.9 per cent.; potassium chlorid (KCl), 0.042 per cent.; sodium bicarbonate ($NaHCO_3$), 0.03 per cent.; calcium chlorid ($CaCl_2$), 0.024 per cent. (Dextrose 0.1 per cent.)

The dextrose was omitted after Experiment 43, until Experiment 123, when it was again used as its absence was found to facilitate spontaneous rhythmic contractions.

The technic described was adequate to detect epinephrin in Ringer-Locke solution in a concentration of 1 to 25,000,000, sometimes of 1 to 50,000,000 or 100,000,000 in cool weather. The sensitiveness is, however, subject to influences not well understood, such as heat, or too many minute's delay in removing the artery from the dead animal. As already mentioned, sodium citrate damages the preparation. The same is true of sodium oxalate, although herudin proved to be innocuous. Therefore, all experiments in which the arterial strip failed to prove its sensitiveness in the presence of the anticoagulant employed were discarded. For this control, usually serum, sometimes platelet extract or epinephrin, was employed. Experiments in which clotting could be demonstrated were also discarded.

PART II

SOURCE OF THE VASOCONSTRICTOR SUBSTANCE

Defibrinated Blood.—Although the effect of defibrinated blood on the excised artery has been noted by many observers, it may be well to illustrate it (Figs. 3, 4, and 13). Its chief characteristics are the immediate onset, the abruptness and the extent of the constriction.

Uncoagulated Blood.—Blood of the Ox.—Blood of the ox was first investigated because it was from the ox that the test-object was obtained.

Method.—Blood of the ox was obtained as follows: A wide-mouth bottle of one liter capacity, and cork, were paraffined. A 3.8 per cent. sodium citrate solution was prepared. This concentration is isotonic (Rous and Turner[33]). As the solution proved to be acid to litmus it was neutralized with dilute sodium hydroxid (NaOH) to a pH of 6.8 to 7.6 (Levy and Marriott[34]). The animals were usually slaughtered by cutting the neck. The bottle, containing a suitable amount of citrate, was held under the stream of blood and filled instantaneously. It was then inverted several times and brought to the laboratory in a pail of cracked ice. Here the volume was measured and the maximum concentration of citrate calculated. This varied from 0.6 to 0.3 per cent. The increase of volume resulting from the addition of citrate was less than one fifth. The mixture was strained through two layers of fine gauze to ascertain the presence of clots. The fluids were tested in the following order.

1. Ringer-Locke solution.
2. Ringer-Locke solution minus calcium.
3. Ringer-Locke solution containing citrate in the same concentration as the blood to be investigated.
4. Uncoagulated (citrated) blood; this was allowed to remain in contact with the artery as long as seemed feasible without damaging it, usually three or four minutes.
5. Defibrinated blood to which citrate in the above concentration had been added after defibrination, the object being to prove that the preparation had retained its sensitiveness in the presence of citrate.

In the case of the herudinized blood the order was as follows:

1. Ringer-Locke solution.
2. Ringer-Locke solution containing herudin in the same concentration as the blood to be tested.
3. Herudinized blood.
4. Defibrinated blood to which herudin in the same concentration had been added after defibrination.

In some of the later experiments herudin was omitted from the defibrinated blood because of its scarcity, its innocuousness with respect to the excised artery having been amply demonstrated.

Results.—Eighteen specimens of citrated ox blood were examined. Of these, eight experiments were discarded because the sensitiveness of the artery was insufficient or unproved, and one because the concen-

33. Rous, P., and Turner, J. R.: Jour. Exper. Med., 1916, **23,** 219.
34. Levy, R. L., and Marriott, W. McK.: The Archives Int. Med., 1915, **16,** 389.

TABLE 1.—CITRATED OX BLOOD

Experiment	Constriction	Citrate, Per Cent.	Material Obtained
61	0	0.5	1 day previous
66	+	0.5	1 day previous
99	+++	0.5	Same day
146	0	0.5	Same day
	0	0.5	Same day
149	0	0.48	Same day
	—	0.62	Same day
152	0	0.3	Same day
	—	0.33	Same day

Explanation of Tables

Under "Constriction" is recorded the reaction of the artery.
+++ = maximal constriction.
++ = marked constriction.
+ = moderate constriction.
Trace = slight constriction.
0 = no constriction.
— = dilatation.
Under "Material Obtained" is recorded the time elapsed between collecting and testing the material.

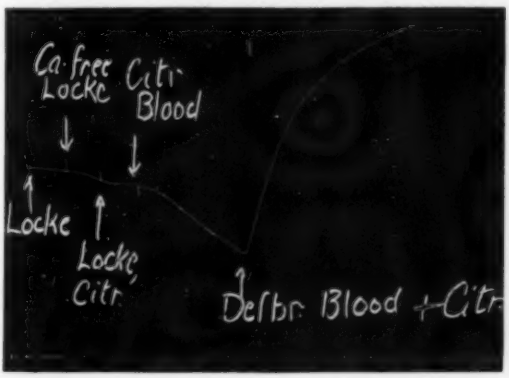

Fig. 1.—Experiment 149. Ox carotid. Citrated ox blood. Order of tests: (1) Ringer-Locke solution; (2) same without calcium; (3) Ringer-Locke solution containing 0.62 per cent. sodium citrate; (4) ox blood containing 0.62 per cent. sodium citrate; (5) defibrinated blood containing same. Time in minutes in all figures except as otherwise indicated.

tration of citrate used (0.25 per cent.) seemed too small. Of the remaining nine specimens, as shown in Table 1, seven caused no change or else a moderate fall of tonus, whereas only two caused a rise. A typical example of the lack of vasoconstrictor action is shown in Figure 1, in which substitution of citrated blood for citrated Ringer-Locke solution caused a moderate relaxation lasting three and one-third minutes, at the end of which the arterial strip reacted vigorously to defibrinated blood to which sodium citrate in the same concentration had

been added. Similarly, in Figure 2, citrated blood caused only a very slight delayed rise followed by a slight fall. At the end of eight and one-half minutes the arterial strip showed extreme sensitiveness by the degree of its response to citrated plasma. In the two cases in which a vasoconstrictor action was observed, no careful examination for clot was made, as was done in the subsequent experiments. Our suspicion that the rise was due to partial clotting was therefore not confirmed. The large proportion of specimens that had no vasoconstrictor effect, however, justifies the conclusion that citrated blood of the ox, as obtained, has no constrictor effect on the excised ox artery.

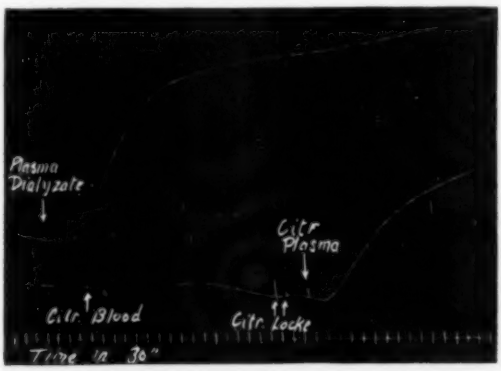

Fig. 2.—Experiment 61. Ox carotid. Ox blood. Upper tracing: Effect of dialyzate of citrated plasma, after three-hour dialysis against Ringer-Locke solution. Lower tracing: Effect of citrated ox blood and of plasma obtained from it.

The foregoing experiments seemed open to criticism because of the possibility of contamination by tissue juices or other substances which might have affected the behavior of the artery. Use of laboratory animals made it possible so to regulate the conditions under which blood was withdrawn, that our scanty supply of herudin could be utilized. This anticoagulant has the advantage that it does not reduce the sensitiveness of the arterial strip. Accordingly, numerous experiments were done with blood of the dog. The method used was as follows:

Method. Uncoagulated Blood of the Dog. (Suggested by Dr. M. T. Burrows.)—Enough 4-ounce wide-mouth bottles, together with stirring rods and cannulas, were boiled in soapy water, washed, soaked in 33⅓ per cent. hydrochloric acid to remove traces of alkali, left to wash at length under the tap, thoroughly washed with distilled water, dried, oiled, and left to drain. The cannulas were made as large as could be forced into the artery. They were about 1 foot long, and suitably bent to reach the bottom of the bottle, below the level of the anticoagulant solution. The carotid artery was exposed and

wiped free of blood by means of a piece of oiled gauze, and further protected from blood by inserting beneath it a similar layer of gauze. It was then nicked, and washed inside, first with paraffin oil, and then (in the later experiments) with strong citrate solution. The blood was then allowed to flow rapidly to the desired volume, with constant stirring. The anticoagulant solutions used were citrate, as in the experiments with blood of the ox, except that it was not neutralized, 1 per cent. oxalate in 0.9 per cent salt solution, and herudin in Ringer-Locke solution. The concentrations are indicated in the tables. Certain modifications of method, such as neutralizing of the citrate solution, observation of sterile precautions, drawing of blood by syringe without anesthesia, use of excessive oxalate later to be reprecipitated, were tried, but did not appear advantageous. For anesthetic, ether was gradually discarded in favor of paraldehyd, which seemed better for our purpose. The order in which the tests were carried out was the same as that already described.

TABLE 2.—Citrated Dog Blood

Experiment	Constriction	Citrate, Per Cent.	Material Obtained	Remarks
96*	Trace	0.2	Same day	Small clot next day
98	+	0.2	Same day	Small clot next day
100	+	0.2	Same day	Artery not very active
100	+	0.5	Same day	Artery not very active
101	Trace	0.2	Same day	Artery not very active
113*	0 ?	0.3	1 day previous	Sensitive artery. No effect in 2½ minutes
113	Trace	0.3	1 day previous	Sensitive artery
116	+	0.3	Same day	Clot ?
123	+	0.2	Same day	

* Brackets indicate more than one observation on same material.

Results.—Oxalated blood of the dog uniformly caused a constriction, which, though marked, was in no way comparable to that induced by defibrinated blood. Citrated blood had a similar effect, though less marked and more variable. Of seven properly controlled experiments, in which the citrated blood was substituted for citrated Ringer-Locke solution, all, as shown in Table 2, showed some increase of tonus varying from a trace to a moderate constriction. The type of curve differs from that caused by defibrinated blood in that the sharp onset and the abrupt rise are replaced by a more or less prolonged latent period and a gradual rise.

Herudinized blood of the dog was investigated in nine experiments, of which two were discarded because of gross clotting, two because of insensitive test objects. The results were not uniform. In Experiment 103, for example, as shown in Figure 3, substitution of herudinized blood for herudinized Ringer-Locke solution failed to cause a rise. The latter solution, however, in itself caused a moderate rise, which may have obscured the results. This was the only occasion in which

the herudin had any such effect. The test object reacted vigorously to defibrinated blood in the presence of the same concentration of herudin, thus demonstrating its sensitiveness. In two experiments the substitution of herudinized blood for herudinized Ringer-Locke solution resulted in no increase of tone for two and ten minutes, respec-

Fig. 3.—Experiment 103. Ox carotid. Dog blood. Effect of herudinized Ringer-Locke solution, herudinized blood, plasma obtained from same by gravity and defibrinated blood to which herudin had been added.

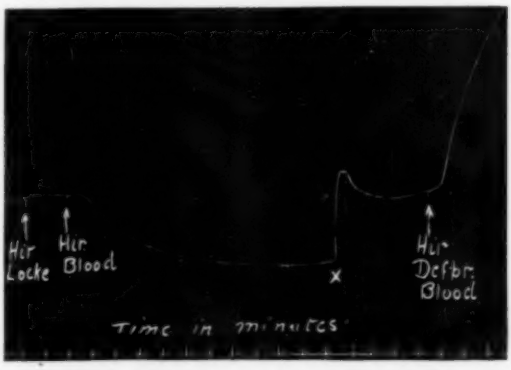

Fig. 4.—Experiment 104. Ox carotid. Effect of herudinized blood of dog as compared to defibrinated blood to which herudin was added. Note spontaneous contractions at X.

tively, and then a sharp rise without any further manipulation. Figure 4 shows the second of these. The herudin blood caused a gradual relaxation lasting for ten minutes, at the end of which period a sharp spontaneous rise (X) occurred, followed by an incomplete fall. The further development of the curve was interrupted for the purpose of testing the artery by the use of defibrinated blood to which a like amount of herudin had been added. As far as it goes, however, this curve is precisely similar in form to rhythmic changes of tonus which

we have since frequently observed in arteries left undisturbed in Ringer-Locke solution containing no dextrose. Certainly no other explanation is at hand for such a prodigious latent period. Two other specimens of blood caused a rise, not explicable in this manner.

DISCUSSION

The presence of a vasoconstrictor substance in many of the specimens of blood which had been withdrawn into anticoagulant solutions may be tentatively explained as follows: After the blood leaves the circulation, and before it becomes thoroughly mixed with the anticoagulant solution, the blood platelets break down and liberate a vasoconstrictor substance. That they break down with extreme ease has been shown by Deetjen.[35] That when broken down they liberate a powerful vasoconstrictor substance is demonstrated later in this paper. Coagulation of blood is accompanied both by elaboration of vasoconstrictor substance and breakdown of platelets. That this breakdown is out of proportion to the amount of clot formed is shown by the fact that a very small clot is associated with a very marked vasoconstrictor action. This observation was made by O'Connor[12] and Kahn,[26] and confirmed by the present work. Thus, there is much indirect evidence that the foregoing explanation is correct. We are therefore inclined to disregard those experiments in which a vasoconstriction occurred, and to emphasize those in which it was absent. From this point of view, the results obtained with uncoagulated blood of the dog tend to substantiate those obtained with uncoagulated blood of the ox.

Blood of the Calf.—After the above conclusions were reached, a final experiment was performed on the blood of the calf. This experiment seems worth a detailed description. It was undertaken to obviate on the one hand the possibility of contamination as in the experiments on the blood of the ox, and on the other hand the uncertainties of using a blood foreign to the species from which the artery was obtained, as in the experiments on the blood of the dog. In addition, it offered the advantage that herudin could be used. The protocol follows:

Experiment 164.—Aug. 16, 1917.

12:30 p. m. Carotid arteries of the ox collected in cold Ringer-Locke solution about one hour previously, arrived at the laboratory.

3 p. m. Calf, 4 days old, bled through an oiled cannula inserted into the carotid artery, according to the method used in obtaining blood from the dog. The artery was washed out inside with citrate solution and oil before inserting the cannula. All possible care was taken, and no technical errors were detected. The blood was obtained in two lots, one containing herudin (4 years old) in the proportion of 1 mg. to 6 c.c. of blood, the other (a product obtained from C. E. Bischoff and Company about three months previously) in proportion of

35. Deetjen, H.: Ztschr. f. physiol. Chem., 1909, **63**, 1.

1 mg. to 2.5 or 3 c.c. of blood. The plasma was obtained by centrifugalization at 1,800 R. P. M. for one-half hour for the tests with Arteries 1 and 2, for one hour for the tests with Arteries 3 and 4. One lot of blood, probably that containing the older herudin in weaker concentration, was found after one-half hour at 38 C. to contain half a dozen small clots. The other lot was free from clots.

9:30 p. m. Beginning of tests. The artery was then in Ringer-Locke solution containing dextrose but no sodium bicarbonate. This fluid was replaced by Ringer-Locke solution containing herudin in the same proportion as the second specimen of blood, 1 mg. to 2.5 c.c. The concentration of herudin in the control solution was therefore equal to or greater than that of the blood tested. The herudinized Ringer-Locke solution was then replaced by herudinized blood, this by herudinized plasma. Finally the artery was tested by means of defibrinated blood.

Results.—The results are shown in Figure 5. Neither the herudinized Ringer-Locke solution, the herudinized blood nor the herudinized plasma had the slightest effect on the artery. Indeed, the tonus

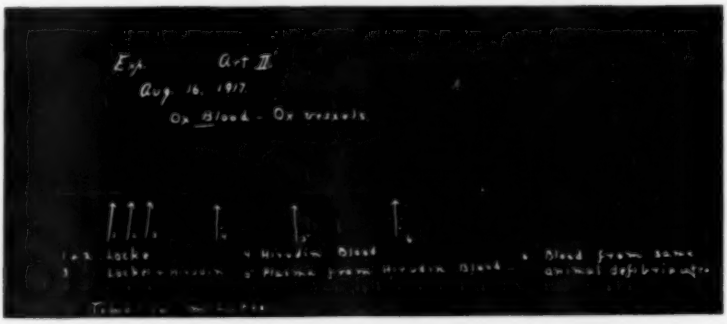

Fig. 5.—Experiment 164. Ox carotid. Calf blood. Effect of herudinized blood and plasma containing no clot. Order of tests: (1) and (2) Ringer-Locke solution; (3) same with herudin; (4) herudinized blood; (5) Plasma from herudinized blood; (6) blood from same animal defibrinated.

remained so constant that it was suspected that the artery was dead. By the use of defibrinated blood, however, a sharp characteristic rise resulted, thus proving the sensitiveness of the preparation. The blood was in contact with the preparation without effect for four minutes, the plasma for five minutes. A second preparation treated with the same materials in the same order gave the same results. In this case the blood was in contact with the preparation for eight minutes, and the plasma for seven minutes. The response to defibrinated blood was even more marked in this test than in the previous one. The blood and plasma contained no clot. In contrast to the foregoing are the results shown in Figure 6. Here substitution of herudinized blood for herudinized Ringer-Locke solution caused very moderate slow rise. On replacing the blood with herudinized plasma the rise continued,

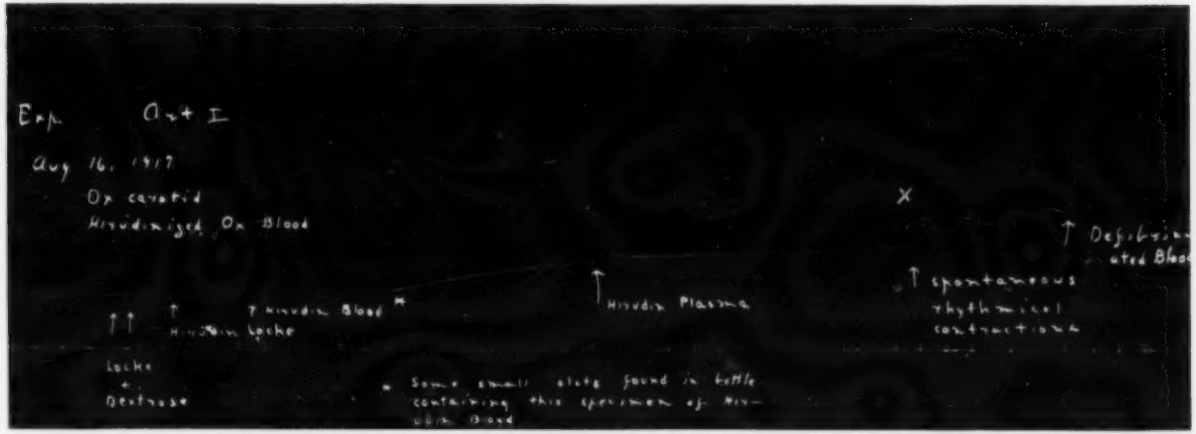

Fig. 6.—Experiment 164 continued. Ox carotid. Calf Blood. Effect of herudinized blood containing clot, and of plasma obtained from it. Order of tests: (1) and (2) Ringer-Locke solution; (3) herudinized Locke solution; (4) herudinized blood in which some small clots were found; (5) herudinized plasma; (6) defibrinated blood. Note spontaneous contractions at X.

and later spontaneous rhythmic contractions developed. The blood in this case contained several small clots. In this experiment, then, blood in which clotting had been entirely prevented had no effect on the artery of the same species, whereas blood in which a small amount of clotting had taken place caused a definite increase of tonus.

To summarize the results obtained with uncoagulated blood:

Citrated blood of the ox as obtained in the slaughter house had no constricting effect on the excised ox artery. Blood of the dog obtained by a method which was less open to criticism had a variable amount of vasoconstrictor action, less in the case of the citrated blood than in the case of the oxalated blood, and nil over a relatively long period in three out of five experiments with herudinized blood. As the latter experiments were not unequivocal, a final experiment was made with blood of the calf, with a clear-cut result that, provided it had been prevented from clotting, it had not the slightest effect on the excised artery. It seems safe to conclude, therefore, that there is no vasoconstrictor substance in the normal circulating blood of animals.

Blood Platelets.—These were obtained according to the method of differential centrifuging first suggested by Mosen[36] and described by Bayne-Jones[37] and Lee and Vincent,[38] by which advantage is taken of the relatively low specific gravity of the platelets.

Method.—Ox blood centrifuged at 1,500 to 1,800 R. P. M. for from a half to three quarters of an hour was found to separate into two approximately equal layers, sediment and plasma. This plasma was aspirated and centrifuged at 3,000 R. P. M. for periods of about five minutes; each time the supernatant fluid was decanted and centrifuged afresh. By repeating this process three or four times a sediment was obtained in which at most two or three cells per high-power field could be detected by microscopic examination of the fresh specimen. The scanty platelet sediment thus obtained, though cohesive, could be emulsified by the use of a capillary pipet, and washed, first, in 0.9 per cent. saline, and finally in Ringer-Locke solution. After two or three washings it was shaken vigorously by hand with glass beads and distilled water, and a volume of double-strength Ringer-Locke solution equal to that of distilled water was added. Out of nearly a liter of citrated blood of the ox a thin film of pure platelet was obtainable, in volume a small fraction of a cubic centimeter.

Five experiments were performed with pure ox platelets, a great number with less pure platelets of ox, and one each with platelets of dog and pig.

Results.—The results were constant and very striking. The effect of the platelet extract is to cause an abrupt rise of the writing-point, which rapidly gathers momentum and continues to rise at a rapid rate until the maximum effort of the artery is expended. This is indicated by the change in shape of the strip, long and attenuated, before the constriction; short and contracted afterward. The effect was even

36. Mosen, R.: Arch. f. Anat. u. Physiol., Physiol. Abt., 1893, p. 352.
37. Bayne-Jones, S.: Am. Jour. Physiol, 1912, **30,** 74.
38. Lee, R. I., and Vincent, B.: The Archives Int. Med., 1914, **13,** 398.

more striking than that occasioned by serum or defibrinated blood. Even an extract from a film of platelets at the bottom of a centrifuge tube, so thin as to be scarcely visible, caused a constriction comparable to that of defibrinated blood. Once contracted, the vessel remained so until treated with nitrite in a manner to be described. The effect is shown in Figure 8 at (3). The abrupt onset, the rapid rise, and the wide latitude of the constriction, comprising almost the entire width of the drum, are well shown. Perhaps the activity of the platelets is best indicated in Figure 11, which demonstrates the effect of a dried preparation of platelets diluted 1 : 100,000 in Ringer-Locke solution. This preparation was obtained by boiling the platelet extract in slightly acid medium and drying the filtrate. With only a single exception, easily explicable on technical grounds, the platelet extract caused this very striking constriction of the excised artery. We were also able to confirm Stewart's[39] observations that the platelet extract causes constriction when injected into the interior of a long piece of artery without coming into contact with the cut surface of the vessel.

Erythrocytes.—In order to free the red corpuscles from platelets, advantage was taken of the low specific gravity of the latter, on the assumption that slow centrifuging through large quantities of fluid would leave the platelets in suspension.

Method.—Accordingly, from 10 to 50 c.c. of citrated blood were poured on top of 0.9 per cent. salt solution in 50 c.c. centrifuge tubes and centrifuged at slow speed for periods of about ten minutes. The speed was such as to require about one hour for separation of ox blood into equal quantities of plasma and sediment. The washing fluid was usually 0.9 per cent. sodium chlorid. Solution of greater specific gravity, such as 2.7 sodium chlorid and 10 per cent. commercial dextrose, were tried and seemed to offer no advantage, nor did preliminary sedimentation by gravity through a large quantity of fluid. After centrifuging, the supernatant fluid was decanted and the sediment poured into a tube of fresh solution. This process was repeated at least four times; the washed corpuscles were examined microscopically by means of Wilson's stain in three experiments. In one, occasional large clumps of platelets; in a second, no typical platelets whatever; in a third only rare ones, were seen. The number of platelets was, therefore, at least very much diminished. The strongest concentration of red corpuscles tested was a 1:3 dilution of a suspension so dense as to be indistinguishable from blood. The cells were laked by shaking with distilled water and glass beads, and brought to physiologic strength by adding a volume of double strength Ringer-Locke solution equal to the distilled water. A similar mixture of distilled water and double strength Ringer-Locke solution was applied to the artery immediately before the test as a control. In all, seven specimens of red blood corpuscles were examined, and the arteries were found to be active in all experiments.

The results are shown in Table 3. In only one experiment, the first, was there more than a slight increase of tone. In this a sharp rise inaugurated a series of rhythmic contractions associated with

39. Stewart, G. N.: Arch. d. mal. du coeur, 1914, **7**, 454.

TABLE 3.—Red Corpuscles Shaken with Water + Double Locke

Experiment*	Constriction	Concentration†	Material Obtained	Remarks
51	++	Thin	1 day previous	Latent rhythm with increased tonus
60	0	1:50	2 days previous	
62	0	Rather thin	1 day previous	
63	0	Dense	Same day	
65	Trace	Medium	1 day previous	Rhythmic contractions with very slight increase of tonus
82	+	Dense	Same day	Belated moderate rise associated with rhythmic contractions
91	0	1:3	Same day	

* The first five experiments were performed with ox corpuscles, the remainder with dog corpuscles.

† Under concentration is indicated the degree of dilution of the corpuscles before testing.

Fig. 7.—Experiment 91. Ox carotid. Dog blood. Order of tests: (1) water and an equal volume of double Locke solution; (2) laked red corpuscles restored to tonicity by means of double strength Ringer-Locke solution; (3) defibrinated blood.

increasing tone, a phenomenon precisely similar to that observed later in a number of instances in arteries left undisturbed in Ringer-Locke solution. The same phenomenon, though much less marked, occurred in Experiment 82 after a latent period of several minutes. In Figure 7 is shown the absence of effect of laked corpuscles of dog in a dilution equal to one-third of a very dense suspension, as compared to the very marked vasoconstrictor effect of defibrinated blood. The results are consistent; red corpuscles of dog or ox, laked or in suspension, have no effect on the excised artery other than a tendency to rouse its latent rhythmicity. The objection might be raised that a vasoconstrictor substance diffused out of the corpuscles during the washing. If so, it should diffuse out into the circulating blood and render the latter more active than it actually is. Moreover, platelets which were treated in a similar way, except that the centrifuging was necessarily faster, remained active after repeated washings.

Leukocytes.—The method followed was essentially that of Zinsser.[40]

Method.—Four dogs were injected, in all instances with a 5 per cent. suspension of aleuronat in bouillon or distilled water, containing 3 per cent. of soluble starch. The water appeared better, if anything, than the bouillon. From 10 to 20 c.c. of this mixture were injected into the pleural cavity in the lower axilla, under sterile precautions. After a period varying from one to nine days, the animal was killed by bleeding. This permitted opening of the pleural cavity without contamination with blood. The exudate was withdrawn by means of an oiled 50 c.c. pipet. The contents of one pleural cavity were divided into two parts, the first being tested unmodified, the second, after treatment with distilled water and double strength Ringer-Locke solution in exactly the way described for the blood platelets. The contents of the other pleural cavity were mixed with strong citrate or oxalate solution and centrifuged. The cells were washed three times in 0.9 per cent. sodium chlorid and once or twice in Ringer-Locke solution. The final sediment was shaken with distilled water and restored to tonicity with double strength Ringer-Locke solution in exactly the way described for the platelets. Smears of the original exudate stained by Wilson's stain showed no platelets whatever, nor did platelet counts, according to the method of Wright and Kinnicutt,[41] very kindly performed for us by Dr. Hirose. An examination of a smear taken from the top of the first sediment after rapid centrifuging also failed to demonstrate platelets. Red corpuscles were present in the first specimens in numbers about equal to the leukocytes, but became much less prevalent in later specimens, so that the sediment had a chalky red appearance. The final specimen contained none. It should be noted that, in a preliminary experiment, aleuronat failed to affect the artery in concentration of 10 per cent.

TABLE 4.—WHITE BLOOD CELL PLEURAL EXUDATE OF DOG

Experiment	Constriction	Concentration*	Red Blood Corpuscles†	Platelets	Remarks
Part 1					
116	0	1:1	++	0	Exudate untreated
123	Trace	1:1	+	0	Exudate untreated
127	0	1:1	+	0	Exudate untreated
Part 2					
116	++	1:5	++	0	Exudate + H_2O + double Locke
123	+—	1:5	+	0	Exudate + H_2O + double Locke
127	0	1:5	+	0	Exudate + H_2O + double Locke
Part 3					
116	+	1:20	++	0	Washed sed. + H_2O + double Locke
117	—	1:20	++	0	Washed sed. + H_2O + double Locke
123	0	1:20	+	0	Washed sed. + H_2O + double Locke
127	0	1:5	0	0	Washed sed. + H_2O + double Locke

* Under concentration is indicated the degree of dilution of the material before testing.
† Under red blood corpuscles is indicated the extent to which red corpuscles were present in the material.

40. Zinsser, H.: Infection and Resistance, The MacMillan Co., New York, 1914.

41. Wright, J. H., and Kinnicutt, R. Jour. Am. Med. Assn., 1911, **56,** 1457.

Results.—In all, seven pleural exudates, taken from the four dogs, were examined. The results are shown in Table 4. Part 1 shows the effect on the ox artery of the exudate untreated; Part 2, that of exudate treated with water and double strength Ringer-Locke; Part 3, that of washed sediment similarly treated. The greatest constriction observed with untreated exudate was a trace. The modified exudate caused a marked constriction in one instance, inconsistent results in a second, and no effect in a third. The washed and treated sediment caused a moderate constriction in the first experiment only; in the other three, no effect or slight loss of tonus.

Although the absence of platelets from all specimens was demonstrated, the absence of broken-down products of platelets was not, since the latter might have been in solution or in a form not recognizable under the microscope. This is especially likely in those specimens which were found to contain blood. Indeed, it is difficult to conceive in what manner erythrocytes could enter a cavity inaccessible to the platelets, which are not only smaller than the red cells, but also, unlike them, possess the power of independent motion (Deetjen[42]). In the successive experiments the number of red cells diminished, and with it the vasoconstrictor power of the exudate. For these reasons we are inclined to emphasize the results of the last experiment, No. 127, on an exudate which was free of red cells. This experiment will therefore be described at length.

Experiment 127.—Nine days previous to this experiment 20 c.c. of a 5 per cent. aleuronat suspension in 3 per cent. aqueous solution of soluble starch were injected into each pleural cavity of a dog. On the day of the experiment the animal was killed by bleeding, and the exudate immediately removed with an oiled pipet. The exudate from the right cavity was centrifuged and washed as already described, the final volume of the compact sediment being 1.25 c.c. This was treated with 5 c.c. water and 5 c.c. double Ringer-Locke solution, the resulting dilution being 1:8. The effect is shown in Figure 8 to be nil, while 0.6 c.c. of platelet sediment of dog, treated in the same way as the washed exudate and with the same amounts, caused a maximal constriction. That is, 1.25 c.c. of exudate had no effect, whereas half as much platelet sediment caused a maximal constriction. Smears and counts failed to demonstrate any platelets. The differential count of the original exudate gave the following percentages:

Mononuclear basophils	1 per cent.
Endothelial cells	4 per cent.
Polymorphonuclear leukocytes	95 per cent.

One red corpuscle was found after prolonged search.

The tabulated results, in conjunction with the foregoing experiment, seem to justify the conclusion that the cells of the pleural exudate of the dog, in contrast to platelets, cause no constriction of the ox artery. How far this is applicable to the cells of the blood is

42. Deetjen, H.: Arch. f. path. Anat., 1901, **164**, 239.

not manifest. Leukocytes of exudates are said to differ morphologically from those of the blood, yet it is obvious that the former can arise only from the blood, assuming that they do not proliferate in the pleural cavity, an unlikely event, which we have not investigated. In any case, no other method of obtaining platelet-free leukocytes in bulk is at hand, apart from the examination of lymph nodes and other organs—a method even more indirect.

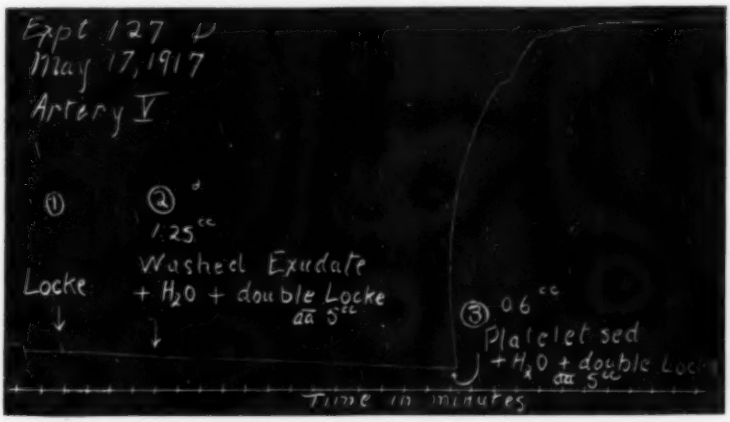

Fig. 8.—Experiment 127. Effect of leukocytes obtained from pleural exudate of dog on ox artery, as compared with effect of dog platelets. Order of tests: (1) Ringer-Locke solution; (2) 1.25 c.c. washed exudate, plus 5 c.c. water, plus 5 c.c. double Locke solution; (3) 0.6 c.c. platelet sediment plus 5 c.c. water plus 5 c.c. double Locke solution.

Tissue Extracts.—

Lymph Nodes.—Eight or ten lymph nodes were obtained from an ox, slaughtered by bleeding, on the day of the experiment. They were freed from fat, chopped, washed once with Ringer-Locke solution, and ground in a mortar with Kahlbaum's washed sand, to which 50 c.c. of Ringer-Locke solution was added. The resulting emulsion was light brown. The pieces of tissue were fairly large, and in spite of the washing it is certain that not all of the blood was removed. In a second experiment the parts of the lymph nodes in which blood was visible were cut away. The remainder appeared free from blood, but the final emulsion was yellowish.

Results.—In both experiments the extract of lymph node caused a vasoconstriction only slightly exceeded by the effect of defibrinated blood.

Other Organs: Similar extracts of spleen, bone marrow, and thymus failed to cause a constriction. The results are difficult to interpret, owing to the complex nature of any organ, together with the difficulty of eliminating the blood. Possibly the cause of the dif-

ference between lymph node and pleural exudate is that the former consists mainly of lymphocytes, the latter of polymorphonuclear leukocytes. It may be mentioned that in a single experiment the lymph of dog gave a marked vasoconstrictor effect, attributed at the time to contamination with blood. Sufficient basis for interpreting the results, however, is lacking.

Plasma.—It was to plasma rather than uncoagulated blood that our attention, like that of most previous workers, was first directed, on the assumption that the effect of the two is identical.

Method.—The plasma was prepared from each of the various kinds of uncoagulated blood mentioned in the section headed "uncoagulated blood," where the method of obtaining the blood has also been described in full. Separation of the plasma from the corpuscles was effected in the earlier experiments by prolonged centrifuging at a speed of 3,000 revolutions per minute, with the idea of getting rid of the blood platelets. As this degree of force was later thought to favor the breakdown of blood platelets, it was reduced, first to a period varying from one-half to one hour at 1,800 R. P. M., later to several hours at very slow speed; and finally to sedimentation under gravity alone, usually overnight. Blood of the ox unfortunately failed to sediment in this manner, but blood of the dog separated very readily. In most of the experiments the centrifuge tubes were oiled or paraffined; this did not, however, seem to offer any advantage over thorough cleaning. In the attempt to obtain a plasma free from vasoconstrictor substance various sorts were investigated, among them citrated plasma of the ox and citrated, oxalated and herudinized plasma of the dog. Plasma of the hen, which had the advantage that it could be prepared without the use of an anticoagulant, simply by keeping the blood cold, was tried on two occasions. Finally a single experiment was made on the plasma of the calf.

In the earlier experiments plasma was compared to Ringer-Locke solution containing the same amount of anticoagulant. In these herudin was not used. Later, when it became evident that the process of preparing the plasma might not be devoid of effect on its activity, it was compared with the uncoagulated blood from which it was obtained. This group of experiments included those on herudinized plasma, and embodied all the technical improvements in the manner of drawing the blood which we could devise. This method has already been described.

Results.—As might be expected, plasma prepared from the type of uncoagulated blood which has been shown to be active, was also active. Citrated and oxalated plasma of the ox and of the dog, when substituted for Ringer-Locke solution containing the same amount of anticoagulant, caused always some increase of tonus. An example is shown in Figure 2. This constriction varied in degree in a seemingly capricious manner from a trace to a well-marked rise. The curves were characterized by the same latent period and gradual onset which were noted in the case of the active specimens of uncoagulated blood. Exchange of plasma for defibrinated blood to which the same amount of anticoagulant had been added resulted almost invariably in a further rise, usually twice to several times the original. It is interesting that plasma of the hen caused a very striking vasoconstriction,

though it is well known that the blood of the hen contains no cells morphologically similar to blood platelets. Plasma of the above types when substituted for Ringer-Locke solution caused a vasoconstriction.

The results of experiments in which the plasma was substituted for the uncoagulated blood from which it was prepared are shown in Table 5, which has been arranged according to the anticoagulant used. In the experiments in which citrate or oxalate plasma was investigated, a definite increase of tonus resulted when plasma was substituted for uncoagulated blood. In the four experiments on herudinized plasma

TABLE 5.—EFFECT OF SUBSTITUTING PLASMA FOR THE BLOOD FROM WHICH IT WAS PREPARED

Experiment	Constriction	Anticoagulant	Concentration of Anticoagulant	Method of Separation
61	++	Citrate	0.3%	Rapid centrifugalization
66	+++	Citrate	0.3%	Slow centrifugalization for 5 hours
108	+	Citrate	0.2%	Gravity + centrifugalization for 1 hour at low speed
108*	Trace	Citrate	0.3%	Gravity
113	++	Citrate	0.3%	Gravity
147	++	Citrate	0.3%	Gravity
92	Continued rise	Oxalate	0.1%	Centrifugalization for 25 minutes at 1,800 R. P. M.
113	Trace	Oxalate	0.09%	Gravity
87	+	Herudin	1:3,300	Centrifugalization for 60 minutes at 1,800 R. P. M.
103	0	Herudin	1:3,300	Gravity
105-B IV	0	Herudin	1:3,300	Centrifugalization 45 minutes at 1,800 R. P. M. and 45 minutes at 3,000 R. P. M.
105-B† V	+	Herudin	1:3,000	Centrifugalization 45 minutes at 1,800 R. P. M. and 45 minutes at 3,000 R. P. M.
164	0	Herudin	?	Centrifugalization 1 hour at 1,800 R. P. M.

* Not very sensitive artery.
† Plasma drawn from a level near the platelet layers.
Experiments 61, 66 and 147 on blood of the ox, the remainder on blood of the dog.

in which this comparison was made, in one, Experiment 87, the plasma had more vasoconstrictor effect than the blood. In this case only was the blood in itself active during the period of observation. In Experiment 105 the plasma drawn from the top of a centrifuge tube caused no constriction, whereas that drawn from a point near the platelet layer did cause a constriction. In the other two experiments no increase of tonus resulted when plasma was substituted for herudinized blood. The first of these is shown in Figure 3 already referred to, in which neither the blood, by replacing herudinized Ringer-Locke

solution, nor plasma by replacing blood, caused any change of tonus in the arterial strip, although the activity of the preparation in defibrinated blood was well marked.

The fourth experiment on herudinized plasma was Experiment 164 performed on herudinized blood of the calf, and has been described at length under the heading "Uncoagulated blood." In Figure 5 it will be noted that, on substituting the herudinized plasma for the herudinized blood from which it was made, no change of tonus whatever was discernible. The preparation then demonstrated its sensitiveness in the presence of defibrinated blood.

To sum up: If a specimen of uncoagulated blood causes a constriction when substituted for Ringer-Locke solution, the plasma prepared from it has the same effect. If, then, plasma from citrated or oxalated blood is compared with uncoagulated blood from which it was prepared, a constriction results. If this same comparison is instituted in the case of herudinized plasma of the dog, the results are variable, but in at least one, and probably two, instances out of three no vasoconstriction resulted. In the experiment on the calf the herudinized plasma, when substituted for the herudinized blood from which it was prepared, had absolutely no effect.

Attempts were made to explain the frequent difference in effect between plasma and uncoagulated blood. Violent and prolonged shaking of the latter failed to increase its vasoconstrictor effect. Comparison between plasma and sediment failed in three properly controlled experiments to show any difference. The most promising way of testing the effect of centrifuging was cut short by the close of work, not, however, before three experiments had been performed. This method consisted in comparing blood, which had been centrifuged and stirred, with the original blood. One experiment showed no increase, a second a slight increase, and a third a marked increase in vasoconstrictor power after this treatment. Further experiments were made to exclude other possible causes of the vasoconstrictor action of plasma. The suggestion was made that the hydrogen-ion concentration was sufficiently altered by the current of oxygen to cause a change of tonus in the artery, and, in fact, the P^H was found by the method of Levy and Marriott[34] to change from 7.25 to 7.45 under oxygenation. This change is much less than that needed to produce a reaction in the artery, and could be prevented by bubbling human alveolar air through the plasma along with the oxygen. Under these conditions the plasma reaction occurred nevertheless. It also occurred, though naturally diminished, in the absence of a current of oxygen. The effect of change of hydrogen-ion concentration, therefore, is excluded. As to the nature of the vasoconstrictor substance which sometimes appears in plasma, it is plausible to assume its identity with that of serum.

For evidence we can only offer the observation, several times repeated, that it passes readily through a celloid membrane (Fig. 2).

Discussion. — If circulating blood is not vasoconstrictor, it is unlikely that the circulating plasma is. Such an event could be explained only on two grounds, either that the blood contains two antagonistic substances, vasoconstrictor and vasodilator, the one in the plasma, the other in the sediment; or that the blood represents a dilution of plasma by means of the inactive cellular (nonplatelet) elements. In either case the sediment should be less active than the plasma. Simpler and more probable is the explanation that centrifuging is by itself sufficient to cause elaboration of a vasoconstrictor substance, most likely by breakdown of platelets. The failure of mechanical shaking to cause this breakdown might be explained on the ground that the intervening red corpuscles prevented the agglutination of platelets which ordinarily precedes their breakdown. The failure to demonstrate a difference in the effect between sediment and plasma, and the occasional increase of vasoconstrictor effect as a result of centrifuging and remixing, afford some evidence that the explanation offered is the correct one, namely, that centrifuging causes an elaboration of vasoconstrictor substance. Very much stronger evidence is offered by the fact that it is possible, by technical improvements and by the use of herudin, to obtain a plasma which, like the blood from which it is prepared, is totally inactive, and in which, consequently, neither the separation of the blood into plasma and sediment nor the mechanical effect of centrifuging can have any influence. The conclusion seems justified, therefore, that the circulating plasma has no effect on the excised strip of ox artery.

PART III

CERTAIN PROPERTIES OF THE VASOCONSTRICTOR SUBSTANCE

Certain physiologic and chemical properties of the vasoconstrictor substance were studied: its effect on the carotid and coronary arteries of the ox, and its relation to heat, precipitation of proteins, dialysis and extraction with various fluids.

Method of Preparation of the Vasoconstrictor Substance.—Blood platelets unmixed with other cells were isolated from the blood of the ox in the manner already described. Enough sediment for the experiments on physiologic effect, heat and dialysis could be obtained in this way. They were treated with distilled water and double strength Ringer-Locke solution as usual. When larger bulk was required, centrifuging for purposes of purification proved too wasteful. Accordingly, a simpler method was used. Two liters of citrated blood were centrifuged for about thirty minutes at 1,800 R. P. M. The cloudy plasma was aspirated and centrifuged for about thirty minutes at 3,000 R. P. M. The resulting plasma was removed by aspiration. The sediment consisted of a gummy white mass discolored with erythrocytes and containing a small

amount of plasma. It was treated in one of two ways. It was shaken with 50 c.c. of distilled water, washed into a beaker and brought to a boil. Dilute hydrochloric acid was added drop by drop until a flocculent precipitate appeared. This precipitate was filtered off and washed with distilled water. The filtrate and washings were dried over a steam bath. The dried product was accumulated and kept in desiccator. From 10 liters of citrated blood about 1 gm. of the dried product was obtainable. Between 0.1 and 1.0 gm. of this dried substance was used for extraction. That this amount was more than ample is shown by the fact that the substance was capable of causing an extreme constriction in a dilution of 1:100,000; that is, for the 5 c.c. volume needed for the physiologic tests, only 0.00005 gm. of the substance was required. Extraction consisted simply in stirring the finely divided dried substance in an evaporating dish by hand at room temperature. The extract was filtered off and the residue washed repeatedly with the extractive. The filtrate was dried on the steam bath. The filter paper and residue were spread out on the electric stove and allowed to dry until no further odor of the extractive could be detected. To both extract and residue distilled water was added and subsequently a volume of Ringer-Locke solution equal to that of the water.

Fig. 9.—Experiment 33.—Upper tracing: Ox carotid. Lower tracing: Ox coronary. Two rings. Lifting weight, 10 gm. Stretching weight, 20 gm. At X, Ringer-Locke solution replaced by aqueous extract of pure platelets of ox, restored to tonicity by the addition of double strength Ringer-Locke solution.

The other method consisted in mixing the platelet sediment with plaster-of-Paris. After the mass had set, it was ground up and extracted by means of the Soxhlet apparatus. Control extractions of plaster-of-Paris yielded no vasoconstrictor substance. Each individual extract represented all the platelets obtainable from not less than 250 c.c. of blood, usually about 500 c.c. of blood. In one experiment, No. 84, platelets of the pig were used; in all the others, platelets of the ox. The manner and duration of the extraction are indicated in the tables. Extracts were taken up with distilled water and double strength Ringer-Locke solution added. Other methods will be described as they come up.

Physiologic Action.—The constrictor action of platelet extract on the carotid artery has already been described. Its action on the excised coronary artery was observed in two experiments.

Method.—For this purpose rings were cut from the coronary artery of a fresh ox heart. They were not cut open, but were tied together and suspended in Ringer-Locke solution. In Experiment 33 two rings were used; the stretching weight was 20 gm., and the lifting weight 10 gm. In Experiment 145 smaller

rings were used and the weights were 10 and 6 gm., respectively. The material for the first experiment was obtained from pure platelets; that of the second from platelets containing an admixture of red cells.

Results.—The effect of the platelet substance on the carotid and coronary arteries is shown in Figure 9, Experiment 33. An immediate well marked contraction of both preparations resulted. In Experiment 145 the effect on the coronary artery was the same. Epinephrin, on the other hand, in a concentration of 1 : 50,000, subsequently caused a sharp relaxation of the same preparation.

Discussion.—The fact that the platelet substance causes a constriction of the coronary artery of the ox, whereas epinephrin, as is well known,[43] causes a relaxation, is strong evidence that the vasoconstrictor substance of platelets is not epinephrin. Blood serum has been shown to have this constrictor effect on the coronary artery of the ox.[1] The similarity of blood serum and platelet substance in this respect substantiates the conclusion stated, that the vasoconstrictor substance of clotted blood is derived from the platelets.

Resistance to Heat.—

Method.—To determine the resistance of the platelet substance or of defibrinated blood to heat, they were boiled. A solution of pure platelet substance in Ringer-Locke's fluid was heated until it boiled vigorously. Again, pure platelets in 50 c.c. of distilled water containing 1 c.c. of 3 per cent. acetic acid were boiled for five minutes, then evaporated to dryness over the steam-bath and extracted with 5 c.c. of Ringer-Locke solution. Defibrinated blood was also brought to a boil and filtered. Moreover, the ordinary process by which the platelet substance was prepared for many of the extraction experiments involved boiling and drying over the steam bath. On other occasions the drying was continued over night, with the result that the remainder was partially carbonized. The products were extracted with Ringer-Locke solution, where necessary, and tested by means of the arterial strip.

Results.—The boiled solutions of pure platelets both caused a vigorous constriction of a pair of arterial strips, that which was boiled the longer causing a maximal constriction. The filtrate of boiled defibrinated blood caused a maximal constriction in a dilution of 1 : 4. The dried heat-acid filtrate caused invariably very marked constrictions, usually maximal, and in a dilution as high as 1 : 1,000,000 (Fig. 11). All specimens of partially carbonized platelet substance caused vigorous constrictions.

Discussion.—That the vasoconstrictor substance both of platelets and of defibrinated blood resists heat substantiates to some degree the conclusion already reached, that its origin is the same. The possibility that the platelet substance is epinephrin is not thereby excluded, as the behavior of the latter under the conditions described was not investigated. The results are not inconsistent, however, with the inference

43. Park, E. A.: Jour. Exper. Med., 1912, **16**, 532.

drawn by physiologic methods, that the vasoconstrictor substance from platelets is not epinephrin.

Relation to Proteins.—Precipitation by Heat and Acid.

Method.—The platelet sediment of about 1 liter of citrated ox blood was mixed with distilled water and precipitated by heat and acid. The precipitate was caught on a filter, drained, extracted with 5 c.c. distilled water and restored to tonicity by means of 5 c.c. of double strength Ringer-Locke solution. Its effect on the excised artery was then observed in comparison with that of blood serum.

Results.—The effect of the heat-acid precipitate on the excised artery strip is shown in Figure 10, Experiment 81. A constriction resulted, which, though immediate and marked, was far from maximal, as was shown by substituting blood serum for the precipitate. This

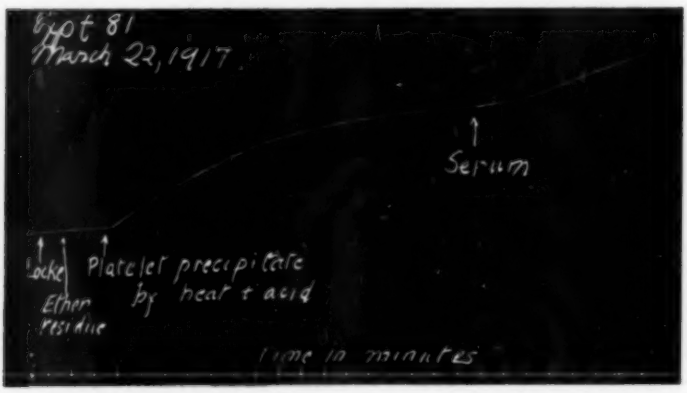

Fig. 10.—Experiment 81. Ox carotid. Ox platelets. Effect of portion of platelet extract coagulable by heat and dilute hydrochloric acid, as compared with effect of ox serum. Ether residue—remainder after evaporation of 50 c.c. ether.

resulted in a further constriction almost equal to the original. This moderate constriction caused by the heat-acid precipitate contrasts strongly with that caused by the dried filtrate obtained by the same process. The latter caused invariably an extreme constriction in numerous experiments, and in a dilution as high as 1:100,000 (Fig. 11).

Discussion.—The activity of the dried filtrate indicates that the vasoconstrictor substance is not precipitated by heat and acid. That the precipitate had any vasoconstrictor action at all might easily be explained on the ground that some of the vasoconstrictor substance was caught mechanically in the flocculent precipitate.

Dialysis.—Celloidin membranes were used in all cases. An active dialyzate in Experiment 36 gave several negative tests for protein. Dialysis was carried on in the refrigerator.

Method.—The material dialyzed was (1) defibrinated blood and (2) pure platelets treated with distilled water and double strength Ringer-Locke solution. These were dialyzed against Ringer-Locke solution in proportions to be indicated. Three experiments were performed on defibrinated blood. In the third, No. 109, an attempt was made to rid the defibrinated blood of vasoconstrictor substance. To this end 20 c.c. were dialyzed against 200 c.c. of Ringer-Locke solution, the dialyzate being changed three times at half-hour intervals, and the dialysis then continued over night, the total duration of the dialysis being twenty hours. The dialyzator was then compared with the

Fig. 11.—Experiment 75. Ox carotid. Ox platelets. Effect of portion of platelet extract not coagulated by heat and dilute hydrochloric acid. Filtrate dried and diluted 1:100,000.

original defibrinated blood as to its vasoconstrictor activity. One experiment, No. 40, was performed on a solution of pure platelet substance, 20 c.c. of which were dialyzed against an equal volume of Ringer-Locke solution for twenty hours. The dialyzate was removed and tested, and then replaced by fresh Ringer-Locke solution. This exchange was repeated at fifteen-minute intervals, five times in all, and the dialyzator remaining in the membrane was tested for vasoconstrictor action.

Results.—The vasoconstrictor substance both of defibrinated blood and of platelets passed through the celloidin membrane very readily. In Experiment 36 a nine-hour dialyzate of 200 c.c. of defibrinated blood against 50 c.c. of Ringer-Locke solution caused a maximal constriction. In Experiment 38 a dialyzate of defibrinated blood caused a maximal constriction after only thirty-five minutes. In Experiment

109 dialysis of defibrinated blood against repeated changes of Ringer-Locke solution so far reduced the activity of the dialyzator that it became only about a third as active as the original defibrinated blood. The effect of the twenty-hour dialyzate of platelet substance is shown in Figure 12, Experiment 40. A marked vasoconstriction resulted. Five subsequent changes of the dialyzate for fresh Ringer-Locke solution had the result that the dialyzator was rendered totally inactive.

Discussion.—The vasoconstrictor substance both of defibrinated blood and of platelet passes readily through a celloidin membrane. The former can be almost completely, the latter completely, removed

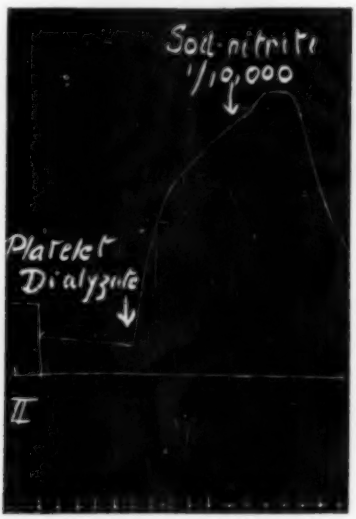

Fig. 12.—Experiment 40. Ox carotid. Aqueous extract of pure platelets of ox restored to tonicity and dialyzed against an equal volume of Ringer-Locke solution. Effect of dialyzate shown. Time in half minutes.

by repeated dialysis. In this respect again defibrinated blood and platelet extract are similar. The rapidity and completeness of the dialysis is further evidence against the identity of the vasoconstrictor substance with protein.

Qualitative Tests.—Several active solutions were tested for protein. The dialyzate of defibrinated blood of Experiment 36 was tested by means of the heat-acid, biuret, potassium ferrocyanid and acetic acid, phosphotungstic acid and sulphuric acid tests. An active extract of platelet substance by means of chloroform and another by means of alcohol were examined with the biuret test. All these tests were

negative. It is possible, therefore, to obtain active vasoconstrictor substance free from protein.

Extraction in Relation to Protein.—By the methods already described, five alcohol extracts, five ether extracts and six chloroform extracts of platelet substance were made. All of the first, three of the second, and four of the third caused definite or marked vasoconstriction. As protein is precipitated by all these fluids,[44] the extracts are protein-free, as was demonstrated in the two qualitative tests described. The presence of vasoconstrictor substance in these extracts is therefore further evidence against its being a protein.

Summary and Discussion.—These results bring out several points in common between the vasoconstrictor substance of platelets and that of defibrinated blood. Both constrict the coronary artery and both resist boiling for a short period. Both dialyze very readily. These results substantiate the conclusion already reached that both substances have the same origin, that is, the platelets. The action on the coronary artery of both substances is diametrically opposed to that of epinephrin. The vasoconstrictor substance of the platelets is not precipitated by heat and acid, dialyzes readily and completely, is present in vasoconstrictor material that is protein-free, and also in numerous active extracts obtained by means of fluids in which protein is insoluble—alcohol, chloroform and ether. It is therefore neither epinephrin nor protein.

Extraction.—The method of preparing the extracts has already been described. That of comparing different fluids with respect to the contents of vasoconstrictor substance follows.

Method.—Since different test objects varied in their sensitiveness, it was necessary to compare the different fluids on the same test object. As most of the constrictions were very marked, and of a type which persisted for an indefinite period, even after washing with Ringer-Locke solution, some method of relaxing the arterial strip to its original tonus was necessary. Sodium nitrite in a concentration of 1:10,000 in Ringer-Locke solution served this purpose. A prompt fall, sometimes after a short further rise, resulted. The preparation was then washed several times with Ringer-Locke solution, and after coming to its original level, was again used. This stimulation of the preparation, first by constricting agents and then by dilating agents, did not impair the sensitiveness of the preparation. The advantage of this method lies in the ability to use the same test objects for successive tests at relatively short intervals of not more than half an hour. Two methods of comparing different substances were used. The first consisted in comparing the vasoconstriction produced by them at the same dilution, a comparison which proved less accurate than the second method unless gross differences were present. The other consisted in making parallel series of dilutions of the two substances Beginning with the highest dilution of the first substance, the dilutions were tested in order until a definite constriction of the arterial strip took

44. Hammarsten, O.: A Textbook of Physiological Chemistry, John Wiley & Sons, New York, 1909, p. 61.

place. Each dilution was washed out with several changes of Locke's solution. Thus what might be called the threshold concentration was determined. This determination was repeated with the second substance. That substance which caused a constriction at the greater dilution was judged the more vasoconstrictor of the two. In order to prove that the sensitiveness of the preparation had not changed, the threshold concentration of the first substance was redetermined. In comparing extracts with residues it was found, however, that dilution by weight and volume gave misleading results, since the extracts contained no salts, whereas the residue contained a relatively large bulk of them. For these reasons comparisons were instituted on the basis of the volume of the blood from which the platelets were obtained. Both extract and residue were made up to this volume, or else to some simple multiple or fraction of it. This method furnished a basis for comparison which was uninfluenced by the amount of impurities in the fluids examined.

TABLE 6.—Absolute Alcohol Extraction of Platelet Substance

Exper.	Constriction	Material	Method	Time	Temperature	Concentration
75	+++	Extract	Evap. Dish	10 min.	Room	1:1,000
	++	Residue	Evap. Dish	10 min.	Room	1:10
92	+++	Extract	Soxhlet	1½ hr.	Warm	Vol. = 10 c.c.
	Trace	Residue	Soxhlet	1½ hr.	Warm	Vol. = 10 c.c.
94	+	Extract	Soxhlet	3 hr.	Warm	1:16*
	Trace	Residue	Soxhlet	3 hr.	Warm	1:1*
114	+++	Extract	Soxhlet	4 hr.	Warm	1:1*
	+	Residue	Soxhlet	4 hr.	Warm	25:1*
161	++	Extract	Evap. Dish	3½ hr.	Room	Diluted 1:2
	++	Residue	Evap. Dish	3½ hr.	Room	Undiluted

* Concentration in terms of original blood volume.

Alcohol Extraction.—Five experiments were performed. The results are shown in Table 6. In Experiment 75, extraction with absolute alcohol at room temperature for ten minutes yielded a product which caused a maximal constriction when diluted 1:1,000 with Ringer-Locke solution, whereas the residue caused a less marked constriction when diluted 1:10. In Experiment 92, extraction for one and one-half hours by means of the Soxhlet apparatus yielded a product which caused a maximal constriction when diluted with 10 c.c. of Ringer-Locke solution, whereas the residue, diluted to the same volume, caused only a trace of constriction. In Experiment 94 similar extraction for three hours yielded an extract which caused a moderate constriction at a concentration of 1:16. The residue caused even less constriction at a concentration as high as 1:1, both concentrations being expressed in terms of original blood volume. The same relation is apparent in Experiment 114, which will be described in detail.

Method.—Defibrinated blood was obtained from the ox. Two liters of citrated blood were also obtained, and divided into two 1-liter portions. From each liter the platelets were isolated by centrifuging in the usual manner. The materials used in the experiment were obtained as follows:

A. Platelets from 1 liter of blood were ground up with plaster-of-Paris and distilled water, and the mass was allowed to dry. It was kept on ice and on

the following day extracted in the Soxhlet apparatus over a water bath, with 50 c.c. of absolute alcohol, for four hours. The extract was then evaporated to dryness on the electric stove under a current of air, and was rather hot when removed. The gummy substance on the dish was taken up with 100 c.c. of Ringer-Locke solution, filtered, and diluted for testing. This was called A. The plaster residue was taken up with 20 c.c. of water, and 20 c.c. of double strength Ringer-Locke solution added. This was called A residue.

B. Platelets from the other liter of blood were kept on ice overnight, then rubbed in a mortar with 50 c.c. of water, then 50 c.c. of double strength Ringer-Locke solution added, and dilutions made for the tests. This was called B.

C. Defibrinated blood was kept on ice over night and diluted for testing. This was called C.

All dilutions were made on the basis of original blood volume. The concentrations are expressed as fractions or multiples of that concentration which resulted when the substance was made up to original blood volume. For example, 1:20 means a concentration 1:20 of this standard, 25:1 means twenty-five time as concentrated, and 1:1 means the same concentration as the standard.

Results.—The results are shown in Figure 13. Defibrinated blood (C) caused a sharp constriction at 1:50. The alcohol residue (A residue) caused a relatively slight constriction at 25:1. The alcohol extract (A) tested in samples of increasing concentration, caused an extreme constriction at 1:1. The platelets (B) tested in a similar fashion, caused a definite constriction at 1:20. Defibrinated blood (C) again caused a sharp constriction at 1:50, showing that the arterial strip had not lost in sensitiveness. Finally, the platelets (B) caused an extreme constriction at 1:10.

The threshold concentrations of the four substances expressed in terms of original blood volume were:

Defibrinated blood	(C)	1:50
Platelets	(B)	1:20
Alcohol extract	(A)	1:1
Alcohol residue	(A res.)	25:1

From these figures it will be seen that the platelets were $\frac{2}{5}$ as active as the defibrinated blood. That is, $\frac{2}{5}$ of the total potential vasoconstrictor power of blood was recovered in the platelets, in spite of the unavoidable loss occasioned by centrifuging. The alcohol extract was $\frac{1}{20}$ as active as the platelets, and the residue $\frac{1}{25}$ as active as the extract.

In Experiment 161 the residue produced about the same constriction as the extract in twice as great a concentration, a result less striking than those enumerated above.

The extracts in all five experiments yielded much more vasoconstrictor substance than the residue. This result was naturally most striking in the experiments in which, as in Experiment 114, the extraction was performed by means of the Soxhlet apparatus, but it was also true in the experiments in which it was performed by stirring at room temperature in an evaporating dish for a relatively short period.

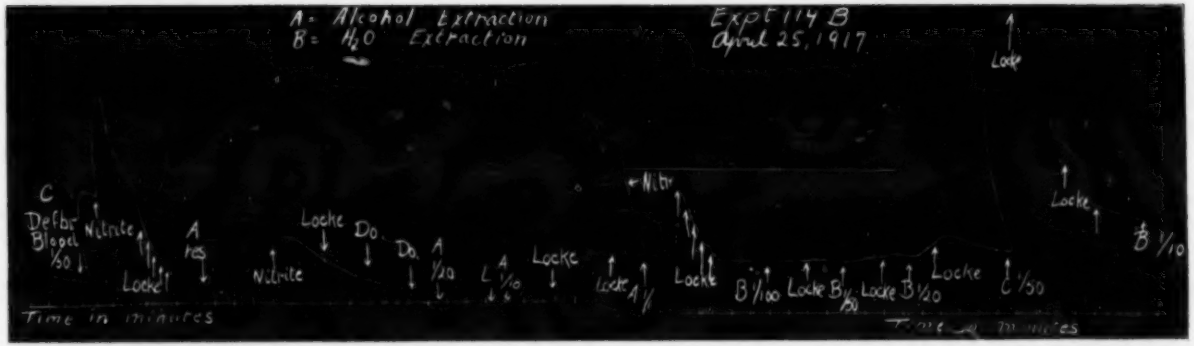

Fig. 13.—Experiment 114-114 B. Ox carotid. Abbreviations used: (C) defibrinated blood; (Nitr.) sodium nitrate 1: 10,000; (A res.) residue after alcohol extraction; (A) alcohol extract; (B) platelets plus water, plus double Ringer-Locke solution.

Ether Extraction.—The results of ethereal extraction are shown in Table 7. In Experiment 80, extraction at room temperature yielded a product which caused a moderate relaxation when diluted with Ringer solution 1:2,500. The dried platelet substance from which this extract was made caused a moderate constriction when diluted the same amount. In Experiment 84 extraction for one hour in the Soxhlet apparatus failed to yield a product which, when diluted with 10 c.c. of Ringer-Locke solution, had any effect on the excised artery. Only after overnight extraction was an active product obtained. The like is true of Experiment 90, in which the extract by means of the Soxhlet apparatus for one hour caused only a trace of constriction. Extract from the same material from the second to the twelfth hour inclusive caused a moderate constriction at a dilution about fifteen times as great. In Experiment 92 the extraction was continued over-

TABLE 7.—ETHER EXTRACTION OF PLATELET SUBSTANCE

Exper.	Constriction	Material	Method	Time	Temperature	Concentration
80	Relaxation	Extract	Evap. Dish	35 m'n.	Room	1:2,500
	+	†				1:2,500
84*	0	Extract	Soxhlet	1 hr.	Warm	10 c.c. Locke
	+++	Extract	Soxhlet	Overnight	Warm	10 c.c. Locke
90	Trace	Extract	Soxhlet	1 hr.	Warm	1:870
	+	Extract	Soxhlet	2-12 hrs.	Warm	1:120,000
92	+++	Extract	Soxhlet	Overnight	Warm	Vol. = 10 c.c.
	++	Residue	Soxhlet	Overnight	Warm	Vol. = 10 c.c.

* Platelets obtained from ⅝ liter of blood in each extraction.
† Original Subs. not extracted.

night and the products diluted with 10 c.c. of Ringer-Locke solution. The extract caused a maximal constriction, the residue a constriction that was almost maximal. The results may be summarized by saying that extraction by ether for a period of an hour or less, whether in an evaporating dish or in the Soxhlet apparatus, yielded little or no vasoconstrictor substance, and that although overnight extraction yielded an extraction somewhat more active than the residue, the activity of the two was of the same order.

Chloroform Extraction.—The results are shown in Table 8. In Experiment 49, extraction of dried platelet substance for a few minutes at room temperature yielded a product which, when dried and diluted with 25 c.c. of Ringer-Locke solution, caused only a trace of constriction. In Experiment 52, a similar process yielded an extract which caused a moderate constriction and a residue which caused a maximal constriction, both being diluted to the same extent. In Experiment 78 the extract, when diluted with 6 c.c. of Ringer-Locke solution, caused only the same amount of constriction as the residue

when diluted with 250 c.c. In these experiments, short extraction at room temperature yielded an extract much less active than the residue. In Experiment 92, extraction by means of the Soxhlet apparatus yielded a product which had no effect on the excised arterial strip when diluted to a volume of 10 c.c., whereas the residue when diluted to the same volume, caused a maximal constriction. As the overnight extraction in this experiment proceeded very slowly, it was continued for one and one-half hours over a boiling water bath. In Experiment 94, extraction by the same apparatus over a water bath for three hours yielded an extract which caused the same constriction as the residue only when five times as concentrated. These results may be summarized by saying that little or no active substance was extracted by chloroform in a period of ten minutes or less, and that the amount obtained by more prolonged extraction was considerably less than that remaining in the residue.

TABLE 8.—Chloroform Extraction of Platelet Substance

Exper.	Constriction	Material	Method	Time	Temperature	Concentration
49	Trace	Extract	Evap. Dish	Minutes	Room	Undiluted
82	+	Extract	Evap. Dish	Minutes	Room	Vol. = 10 c.c.
	+++	Residue	Evap. Dish	Minutes	Room	Vol. = 10 c.c.
78	++	Extract	Evap. Dish	10 minutes	Room	Vol. = 6 c.c.
	++	Residue	Evap. Dish	10 minutes	Room	Vol. = 250 c.c.
92	0	Extract	Soxhlet	Overnight	Warm	Vol. = 10 c.c.
	+++	Residue	Soxhlet	Overnight	Warm	Vol. = 10 c.c.
94	0	Extract	Soxhlet	3 hours	Warm	1:2*
	++	Extract	Soxhlet	3 hours	Warm	5:1*
	++	Residue	Soxhlet	3 hours	Warm	1:1*

* Concentration in terms of original blood volume.

Qualitative Tests for Cholesterol.—In Experiment 94, referred to in Tables 6 and 8, two extracts were tested for cholesterol by means of the acetic anhydrid—sulphuric acid (H_2SO_4) test. The first, a chloroform extract, contained a bare trace of cholesterol, estimated at 0.00001 gm.; the second, an alcohol extract, although ten times as powerful, gave an even fainter reaction for cholesterol.

Summary.—The readiness with which the vasoconstrictor substance is extracted by means of alcohol, contrasted with the difficulty with which it is extracted by chloroform and ether, throws some light on its chemical nature. It almost certainly excludes it from the group of cholesterol and allied substances. This statement receives support from the qualitative tests for cholesterol. The possibility that the alcohol in the Soxhlet experiments was warm enough to extract cholesterol, does not vitiate this conclusion, because even at room temperature a relatively short period of extraction with alcohol sufficed to dis-

solve the greater part of the vasoconstrictor substance. That the results failed to show a sharp distinction between the action of the two types of extractives may be explained in one of two ways: either more than one vasoconstrictor substance is present in the platelets, or the solubilities of a single substance are modified by the presence of a complex group of impurities.

PART IV

RELATION OF THE VASOCONSTRICTOR SUBSTANCE TO THE FACTORS CONCERNED IN THE COAGULATION OF THE BLOOD

Although our study of this phase of the subject was partly incidental to other observations and cannot be regarded as complete, it seems worth recording. It was considered from the point of view, first, of the time relation of the elaboration of the vasoconstrictor substance to the formation of the blood clot; second, of the effect of certain of the factors concerned in coagulation; and third, of the effect of heat on the vasoconstrictor substance as compared to the effect of certain of the factors concerned in coagulation. In discussing these factors the terminology of Howell's theory[45] will be used.

Method.—To determine whether or not the elaboration of the vasoconstrictor substance preceded the formation of clot, a specimen of blood containing no anticoagulant was withdrawn from the carotid artery of the dog and brought into contact with an arterial strip within thirty seconds, and the effect noted. Absence of coagulation was judged by the fact that the fluid could still be aspirated through a small glass tube. In another experiment the effect of gross coagulation on the arterial strip immersed in an avian plasma was observed, the onset of coagulation being judged by the cessation of the bubbles of oxygen. In three experiments the effect of citrated blood of the dog on the arterial strip was noted in connection with very careful examination for macroscopic clots. Of the coagulation factors, thromboplastin was obtained by making tissue extracts. Kephalin was prepared from the brain of the dog. The larger vessels were dissected away, and the brain ground in a mortar with sand (Kahlbaum) in the presence of distilled water. Double strength Ringer-Locke solution was then added in volume equal to that of the distilled water, and the mixture was filtered. Extracts were made by means of Ringer-Locke solution of brain, spleen, and bone marrow, of the dog and of thymus and lymph nodes of the ox. The thrombin was very kindly furnished by Dr. Howell. Prothrombin was obtained from plasma of the dog by Howell's method.[45] The brain extract was tested for thromboplastic activity on one occasion and found to coagulate herudin plasma. The thrombin was also tested and found to coagulate both herudin blood and plasma. No systematic tests of the activity of the coagulation factors was undertaken. In regard to the effect of temperature, the behavior of the platelet extract with respect to heat has been described in Part III, and that of the coagulation factors has been studied by other investigators.

Results.—That the elaboration of the vasoconstrictor substance precedes the formation of macroscopic clot is shown by an experiment

45. Howell, W. H.: Am. Jour. Physiol., 1911, **29**, 187.

in which a specimen of blood was withdrawn from the carotid artery of the dog without the use of anticoagulant, and placed in contact with the artery preparation within thirty seconds. An abrupt powerful constriction resulted. That coagulation was not yet complete was shown by the fact that after the writing point had risen 2 inches on the drum, removal of the blood by aspiration through a narrow tube was accomplished. Again, an avian plasma, obtained by centrifuging blood of the hen in the cold, caused a great but gradual constriction which lasted for several minutes before the completion of coagulation was shown by the cessation of the flow of oxygen. This event was without further influence on the curve. Moreover, in three experiments in which citrated blood of the dog caused a definite, though moderate, constriction, subsequent examination of the blood by passing it through a fine sieve demonstrated that macroscopic clotting had not yet occurred. These observations indicate that elaboration of vasoconstrictor substance precedes the development of the clot and are in accordance with those already cited by Kahn[26] and Stewart and Zucker.[19]

As to the factors concerned in coagulation, a single experiment on prothrombin showed that it failed to influence the excised artery. The same was true of a mixture of thrombin with brain extract. Thromboplastin as obtained from brain, spleen, and thymus produced either no effect or else a slight relaxation, and extracts of lymph nodes alone had any vasoconstrictor effect. The elaboration of vasoconstrictor substance, then, precedes the development of a macroscopic clot, and, on the other hand, the coagulation factors tested failed to cause vasoconstriction. The resistance of the vasoconstrictor substance to heat has already been described.

Discussion.—Of the factors concerned in coagulation, fibrinogen, prothrombin and antithrombin are all present in the normal circulating blood, which, as our experiments almost certainly demonstrate, contains no vasoconstrictor substance. Therefore, these factors have no vasoconstrictor effect in the proportions in which they occur in the circulating blood, and are thus unlikely to have any effect separately. None of them is a crystalloid, whereas, we have shown the vasoconstrictor substance to be crystalloid. Further, none of them is thermostable. Fibrinogen is precipitated at 60 C. (Howell[46]). Prothrombin extracts heated to 60 C. undergo a marked diminution of their activity (Howell[49, 46]). Antithrombin is destroyed at 80 C.[45] Of the factors evolved in shed blood, thrombin, though resistant in aqueous solutions, is destroyed by boiling for one minute in the presence of 1 per cent. sodium chlorid (Howell[47]). Thromboplastin alone, of all

46. Howell, W. H.: The Archives Int. Med., 1914, **13,** 76.
47. Howell, W. H.: Am. Jour. Physiol., 1914, **36,** 1.

the factors concerned in coagulation, resists heat. Thus Howell[48] obtained a splenic extract, the thromboplastic action of which was not affected by boiling. The fact that blood platelets, as demonstrated by Bayne-Jones[47] and confirmed by Howell,[49] contain both prothrombin and thromboplastin, suggests the identity of the latter with the vasoconstrictor substance. This suggestion receives support from the work of Lee and Vincent,[50] who observed that the thromboplastic activity of platelets was not destroyed in fifteen minutes at 120 C. That the vasoconstrictor substance is identical with the thromboplastic substance of blood platelets is therefore possible. The fact, however, that thromboplastin is present in all the tissue extracts mentioned, whereas the vasoconstrictor substance is found only in extracts of lymph nodes and of platelets, renders that possibility remote. Solely from the behavior of the respective substances toward heat, the nonidentity of vasoconstrictor substance with any of the clotting factors may be inferred, and this conclusion is strengthened by our other observations already mentioned. It may be concluded that the vasoconstrictor substance has no probable relation either to the actual formation of the blood clot or to the factors concerned in coagulation.

CONCLUSIONS

1. Uncoagulated blood of the ox or calf has no constrictor action on the excised strip of the ox carotid.

2. Blood platelets of ox, dog or pig yield an extract which has a powerful vasoconstrictor action.

3. Erythrocytes yield no vasoconstrictor substance.

4. Leukocytes, as represented by the cells of the pleural exudate of the dog, yield none.

5. Plasma can be obtained free of vasoconstrictor substance. Its constrictor action, when present, is probably due to changes occurring after it leaves the blood vessels. The circulating plasma of normal animals has probably no such vasoconstrictor action.

6. The vasoconstrictor substance (or substances) is neither protein nor epinephrin. It is a crystalloid more readily extracted by water or alcohol than by ether or chloroform, and is probably not related to cholesterol.

7. The vasoconstrictor substance, though present in coagulated blood, is not dependent on the actual formation of the blood clot, nor is it related to any of the factors concerned in coagulation, with the possible exception of thromboplastin.

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48. Howell, W. H.: Am. Jour. Physiol., 1912, **31,** 1.
49. Howell, W. H.: Am. Jour. Physiol., 1914, **35,** 474.
50. Lee, R. I., and Vincent, B.: The Archives Int. Med., 1914, **13,** 398.

RELATION BETWEEN THE PLATELET COUNT OF HUMAN BLOOD AND ITS VASOCONSTRICTOR ACTION AFTER CLOTTING*

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The effect of defibrinated blood and of blood serum in producing contraction of arterial smooth muscle, either in a perfused organ or in a surviving artery suspended in a chamber, has long been observed. It was the cause for many erroneous reports of the finding of epinephrin in the circulating blood until the work of O'Connor[1] showed clearly that the vasoconstrictor property is not due to epinephrin, but is developed in connection with the process of coagulation and is not possessed by blood kept fluid by anticoagulants. O'Connor's conclusions were confirmed and extended by Stewart,[2] Schultz,[3] Stewart and Harvey,[4] and Janeway and Park.[5] While this demonstration is of importance clinically, chiefly because it discredits all previous work on the supposed detection of epinephrin in shed blood, and invalidates all conclusions therefrom as to the cause of arterial hypertension, nevertheless the problem of the origin and nature of the vasoconstrictor property of shed blood has an independent interest. Its solution may not only facilitate studies on the properties of blood in hypertensive patients, but may have practical importance in connection with the purpuric diseases and the disturbances of the highly complex changes concerned in the coagulation of the blood.

The studies of Janeway, Richardson and Park,[6] reported in this journal, make certain that this vasoconstrictor property is developed chiefly, if not solely, in connection with the disintegration of the blood platelets. The importance of the platelets as a source of the vasoconstrictor substance had already been recognized by Zucker and Stewart[7] and by Le Sourd and Pagniez,[8] but without convincing dem-

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1. O'Connor, J. M.: München. med. Wchnschr., 1911, **58,** 1439; Ibid., Arch. f. exper. Path. u. Pharm., 1912, **67,** 195.
2. Stewart, G. N.: Jour. Exper. Med., 1911, **14,** 377.
3. Schultz, W. H.: Bull. Hygienic Lab., U. S. Public Health Service, 1912.
4. Stewart, H. A., and Harvey, S. C.: Jour. Exper. Med., 1912, **16,** 103.
5. Janeway, T. C., and Park, E. A.: Jour. Exper. Med., 1912, **16,** 541.
6. Janeway, T. C., Richardson, H. B., and Park, E. A.: This issue, p. 565.
7. Zucker, T. F., and Stewart, G. N.: Zentralbl. f. Physiol., 1913, **27,** 85.
8. Le Sourd, L., and Pagniez, Ph.: Compt. rend. Soc. de biol., 1914, **1,** 587.

onstration. In connection with the work reported by Janeway, Richardson and Park,[8] the experiments which follow were carried on in an endeavor to add a further link to the chain of evidence connecting the blood platelets with the development of vasoconstrictor properties by shed blood.

The experiments consisted in the comparison of the degree of vasoconstriction produced in the same ox artery by two specimens of defibrinated human blood of known platelet content. The strength of vasoconstrictor action was estimated in two ways: (1) By the form and height of the curve written on the drum by the lever connected with the artery; (2) by determining the extinction point of the vasoconstrictor action in successive dilutions of the blood; that is, the threshold concentration of vasoconstriction. Details of the various methods employed follow.

So far as I have been able to discover, this is the first systematic comparison of platelet count with vasoconstrictor effect. It is true that Sakai and Hiramatsu[9] compared the blood serum of patients suffering from beriberi with that of normal persons, using as a standard the effect of a known concentration of epinephrin. In this disease they found the blood platelets to average three times the normal. They undertook, however, no systematic count of the blood platelets of those patients whose serum they examined. With reference to the subject of this paper, their work may be regarded as suggestive rather than conclusive.

METHODS

Of the methods employed for the clinical estimation of the number of blood platelets in the circulation, that of Wright and Kinnicutt[10] seemed the most practicable. The principle of this method is the use of special staining solutions in conjunction with the ordinary apparatus used in estimating the red corpuscles of the blood. These solutions are two in number: (1) potassium cyanid in 1:400 solution; and (2) cresyl blue in 1:300 solution. These were kept separately on ice and mixed in the proportion of 3:2 immediately before counting. The mixture was filtered. A drop of blood was taken from the ear of the patient by means of a needle and drawn into a 1:100 diluting pipet, such as is used in the ordinary estimation of red corpuscles. The special solution was then drawn up to the 101 mark and the counting was carried out as for red corpuscles. The effect of this solution was to remove the ordinary cells and leave stained platelets. The Thoma-Zeiss apparatus was used as a counting chamber. As the special thin cover glasses recommended by Wright and Kinnicutt for counting of platelets were not available, those furnished by Zeiss were used. In counting, certain precautions were observed. The cells were counted as soon after mixing as possible. Dust was carefully avoided, as its particles are often the same size as the platelets, although usually distinguishable by their dark staining and irregular outlines. Results were checked in every case by a second count. The estimations were made on the day of the experiment.

The test object used in these experiments is a modification of Meyer's excised

9. Sakai, S., and Hiramatsu, T.: Mitt. a. d. med. Fak. d. kais. Univ. zu Tokyo, 1914, **13,** 177.

10. Wright, J. H., and Kinnicutt, R.: Jour. Am. Med. Assn., 1911, **56,** 1457.

strip of the ox carotid. It has been described in detail in the paper by Janeway, Richardson and Park.[5] A brief description is sufficient here. From the carotid artery of the ox a ring was cut. This ring was cut open, ligatured at each end, and suspended in Locke's solution in such a way that the tension was exerted on the circular muscle fibers. The upper end of this strip was attached to the short arm of a lever, the long end of which wrote on a drum with a magnification of 15½. Through the solution a constant stream of oxygen was kept bubbling. Artery and solution were contained in a small test tube which was suspended in a large water bath maintained at 38 C. by means of an electric thermostat. Through a tube fused to the bottom of the test tube the fluid could be aspirated, and then replaced from above by means of an ordinary pipet. This arrangement permitted complete exchange of fluid with a minimum of mechanical disturbance. The arteries were collected in ice-cold Locke's solution and kept on ice until needed. The excised strip was stretched with a weight of 90 gm. for twenty minutes, at the end of which the weight was reduced to a load of 30 gm. for the experiment. It was then allowed to come to equilibrium.

Twenty c.c. of blood were withdrawn from the vein of the patient's arm by means of a needle and syringe. The blood was defibrinated immediately by beating it with a rubber brush in an evaporating dish. In each experiment comparison was made between the blood of two patients with reference to their platelet count; a blood of high platelet count with one of low platelet count. Since different test objects varied in their sensitiveness, it was necessary to compare the different specimens on the same test object. The obvious method of so doing, that is, comparison of the degree of constriction produced by the two specimens, having proved unreliable, another method was adopted. Parallel series of dilutions were made of the two specimens. Beginning with the highest dilution of the first specimen, the dilutions were tested in order until a definite reaction on the part of the arterial strip took place. Each specimen was washed out by three changes of Locke's solution. Thus, what might be called the threshold concentration was determined. This determination was repeated with the second specimen. That specimen which caused a constriction at the higher dilution was judged the more vasoconstrictor of the two. In order to make sure that the sensitiveness of the preparation had not changed, the threshold concentration of the first specimen was redetermined. By this means a comparison of vasoconstrictor effect was attained which was far more accurate than any attempt to judge the degree of constriction produced by the undiluted blood.

RESULTS

Fourteen experiments were performed, in which blood from twenty-eight patients was examined. The results are indicated in Table 1. Perhaps the meaning of the table will be best illustrated by an example, say Experiment 8. In this, blood from a patient whose platelets numbered 205,000 caused a definite constriction in a dilution of 1:8. Blood from another patient whose platelets numbered 248,000 caused a constriction in the same dilution. Therefore, both samples had about the same vasoconstrictor effect. Again, in Experiment 6, blood from a patient whose platelets numbered 51,000, diluted with an equal volume of Locke's solution, gave no constriction, whereas blood from a patient whose platelets numbered 491,000 gave a very marked constriction when diluted with two volumes of Locke's solution. In this case the second sample had much the greater constrictor effect. If, then, this table is examined with reference to the degree of

TABLE 1.—DATA OF EXPERIMENTS

Experiment No.	Date	Source of Blood: Med. No.	Source of Blood: Diagnosis	Platelet Count	Dilution	Degree of Constriction
I	5/23/17	38142	Pernicious anemia	85,000	1:25 1:10	∓ +
		37838	Syphilis (W.); aneurism of arch of aorta	262,000	1:25	+
II	6/ 4/17	38070	Syphilis (W.); syphilis of aorta; dilated aortic arch; aortic insufficiency; myocardial insufficiency	270,000	1:10 1:5	∓ +
		37860	Carcinoma of stomach; tuberculosis of pleura	867,000	1:10	+
III	6/11/17	37961	Cirrhosis of liver	96,000	1:5 1:1	— —
		38000	Chronic nephritis (?); hypertension	235,000	1:25	+
IV	6/25/17	38113	Chronic myeloid leukemia	332,000	1:2	+
		38028	Chronic myeloid leukemia	868,000	1:4	+
V	7/ 9/17	38130	Syphilis (W.); secondary anemia	63,000	1:1	—
		38215	Chronic infectious arthritis; pyorrhea alveolaris; intestinal parasitism (Ascaris lumbricoides)	218,000	1:2	+++
VI	7/23/17	38551	Pernicious anemia	51,000	1:1	—
		38285	Pulmonary tuberculosis; pyorrhea alveolaris; visceroptosis	491,000	1:2	+++
VII	9/14/17	Genl. No. 119451	Myeloid leukemia	48,000	1:1	+
		38479	Pellagra ?	283,000	1:2	+++
VIII	9/19/17	Genl. No. 120097	Pernicious anemia	205,000	1:8	+
		38484	Arteriosclerosis; hypertension; myocardial insufficiency; chronic nephritis	248,000	1:8	+
IX	10/ 9/17	Genl. No. 119451	Myeloid leukemia	33,000	1:1	—
		Genl. No. 120268	Chronic nephritis	246,000	1:2	+++
X	10/12/17	Genl. No. 120652	Pernicious anemia	81,000	1:4 1:2	— +
		38628	Polycythemia; cerebral thrombosis; hemiplegia (right); aphasia	346,000	1:2	++

TABLE 1.—DATA OF EXPERIMENTS—(*Continued*)

Experiment No.	Date	Source of Blood: Med. No.	Source of Blood: Diagnosis	Platelet Count	Dilution	Degree of Constriction
XI	10/17/17	Genl. No. 120680	Chronic nephritis; secondary anemia	189,000	1:8	—
					1:4	+
		38628	Polycythemia	314,000	1:1	+
XII	10/30/17	Genl. No. 38636	Pernicious anemia	47,000	1:1	—
		120781	Acute nephritis with purpura	180,000	1:2	+++
XIII	11/ 2/17	Genl. No. 120597	Pernicious anemia	142,000	1:8	+
		Genl. No. 121133	Banti's disease	439,000	1:8	+
XIV	11/ 6/17	Genl. No. 121183	Cirrhosis of liver	39,000	1:2	+
		Genl. No. 121133	Banti's disease	302,000	1:2	+

Degree of constriction indicated by plus and minus signs: — = no constriction; ∓ = constriction of one preparation and not of another; + = definite constriction; ++ = moderate constriction; +++ = marked constriction.

Under dilutions the figures indicate the number of volumes of blood in relation to the number of volumes of Locke's solution used in diluting. Thus, 1:1 means 1 volume diluted with 1 volume of Locke's solution.

constriction as compared to the amount of dilution, it will be seen that blood from patients of a higher platelet count caused constriction at a higher dilution than blood taken from patients of a low platelet count; that is, in the former case, the threshold concentration is less. In Experiment 11 only was the lower platelet count associated with the greater constriction. In this experiment the difference in platelet count was relatively slight, 189,000 as compared to 314,000. In Experiments 13 and 14, bloods of different platelet count caused the same constriction. Out of fourteen experiments, then, eleven show a direct proportion between platelet count and constrictor effect, one experiment only shows the reverse, and two experiments are equivocal.

Examples of the curves obtained are shown in Figures 1 to 4. In Figure 1, blood from two patients was compared, the first having an abnormally high platelet count (868,000), the second a normal count (332,000). Neither blood caused a definite constriction when diluted with eight parts of Locke's solution, as seen on the curves at "II⅛" and "I⅛." These figures indicate that one volume of blood was diluted with eight volumes of Locke's solution. The blood of high platelet count, Blood II, caused a definite constriction at a dilution of 1:4; Blood I, of lower platelet count, not until a dilution of 1:2 was reached. Also the greater constriction caused by the former blood at each dilution is evident at a glance. This figure exemplifies the greater vasoconstrictor effect associated with a platelet count above

normal. In Figure 2, Experiment 5, blood of an abnormally low platelet count (63,000) is compared with blood of a normal platelet count. The former had no effect when diluted with an equal volume of Locke's solution as shown under "I1/1," whereas the latter caused a very marked constriction in a dilution of 1:2, as shown under "II1/2." A second test of the first blood was again without effect. This figure illustrates the lesser vasoconstrictor activity of a blood of lower platelet count. In Figure 3 the platelets show a similar relation, and the curves almost exactly duplicate those of the preceding figure. In

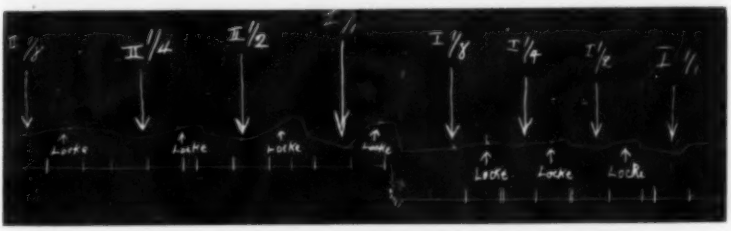

Fig. 1.—Experiment IV. Effect of defibrinated blood in increasing concentration. The fractions indicate the degree to which the blood was diluted. Blood II: Defibrinated blood from a patient whose platelets numbered 868,000 per c.mm. Blood I: Defibrinated blood from a patient whose platelets numbered 332,000 per c.mm. Time: minutes.

Fig. 2.—Experiment V. Blood I: Blood platelets, 63,000. Blood II: Blood platelets, 218,000. Time: minutes and half minutes (clock out of order).

Figure 4 it is demonstrated that bloods of approximately the same platelet count yield curves almost exactly parallel. In fact, the curves produced by Blood II might be fairly accurately superimposed on those of Blood I. The same parallelism was observed with each of two different artery preparations. The figure further exemplifies the fact that the relation between the effects of two samples of blood remained the same with a second test-object as with the first.

Although, as already stated, different test objects vary to such an

extent that no exact normal standard for the threshold concentration is possible, nevertheless a certain rough normal standard can be observed. Blood taken from patients whose platelets numbered less than 100,000 was distinctly below this standard. Nine such specimens were examined. Of these, five caused no constriction when diluted

Fig. 3.—Experiment VI. Blood I: Blood platelets, 51,000. Blood II: Blood platelets, 491,000. Time: minutes.

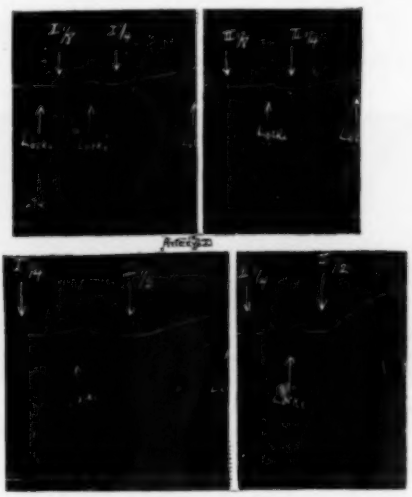

Fig. 4.—Experiment VIII. Showing the effect of the same materials on two different preparations, Artery I and Artery II. Blood I: Blood platelets, 205,000. Blood II: Blood platelets, 248,000. Time: minutes.

with an equal volume of Locke's solution. The platelet counts in these five experiments were 95,000; 63,000; 51,000; 33,000; and 47,000. In Table 2 are shown the platelet counts of those cases which

TABLE 2.—Comparison of Platelet Counts

Experiment No.	Blood Giving No Constriction		Blood Giving Constriction	
	Diagnosis	Platelet Count	Diagnosis	Platelet Count
I	.....	.....	Pernicious anemia	85,000
			Syphilis (W.); aneurysm of arch of aorta	262,000
II	.....	.....	Syphilis (W.); syphilis of aorta; dilated aortic arch; aortic insufficiency; myocardial insufficiency....	270,000
			Carcinoma of stomach; tuberculosis of pleura	867,000
III	Cirrhosis of liver.....	95,000	Chronic nephrtis (?); hypertension..	235,000
IV	.....	.....	Chronic myeloid leukemia.....	352,000
			Chronic myeloid leukemia.....	809,000
V	Syphilis (W.); secondary anemia.....	63,000	Chronic infectious arthritis (spine); pyorrhea alveolaris; intestinal parasitism (Ascaris lumbricoides)	218,000
VI	Pernicious anemia.....	51,000	Pulmonary tuberculosis; pyorrhea alveolaris; visceroptosis	491,000
VII	.....	.....	Myeloid leukemia	48,000
			Pellagra (?)	283,000
VIII	.....	.....	Pernicious anemia	205,000
			Arteriosclerosis; hypertension; chronic nephritis; myocardial insufficiency	248,000
IX	Myeloid leukemia.....	33,000	Chronic nephritis	246,000
X	.....	.....	Pernicious anemia	81,000
			Polycythemia; cerebral thrombosis; hemiplegia (right); aphasia.....	346,000
XI	.....	.....	Chronic nephritis; secondary anemia	189,000
			Polycythemia	314,000
XII	Pernicious anemia.....	47,000	Acute nephritis with purpura.....	180,000
XIII	.....	.....	Pernicious anemia	142,000
			Banti's disease	439,000
XIV	.....	.....	Cirrhosis of liver.....	39,000
			Banti's disease	302,000

caused no constriction in the dilution indicated, as compared to those that did. Only three specimens produced any constriction of the artery when diluted with two volumes of Locke's solution, and only four when diluted with equal volumes. Two of these were from cases of pernicious anemia, one from a case of cirrhosis of the liver, and the fourth from a case of myeloid leukemia. Reference to Table 1 will show that, in general, specimens of bloods of a high platelet count caused constriction to a much greater degree or at a much higher dilution.

No relation could be detected between the type of disease and the degree of vasoconstriction produced by the defibrinated blood. In other experiments blood which had the property of digesting its own clot was tested after the autolysis. No constriction resulted.

SUMMARY

Fourteen experiments with twenty-eight different samples of defibrinated human blood of known platelet count have been described. These experiments were performed by bringing the defibrinated blood into contact with the surviving carotid of the ox suspended according to Meyer's method and registering its degree of constriction on a revolving drum by means of a magnifying lever.

In these experiments bloods of about equal platelet counts produced approximately the same degree of vasoconstriction. With one exception, bloods of higher platelet count produced more marked constriction than bloods of much lower platelet count. This one exception was the blood from a case of polycythemia with a count of 314,000, compared with blood from a case of chronic nephritis with a count of 189,000. The difference in platelet content here is relatively slight.

Of nine specimens of blood with a platelet count less than 100,000, only three produced any constriction of the artery when diluted with two volumes of Locke's solution, and only four when diluted with equal parts of Locke's solution. Two of these were from cases of pernicious anemia, one from a case of cirrhosis of the liver, and the fourth from a case of myeloid leukemia.

CONCLUSIONS

1. The vasoconstrictor effect of defibrinated human blood on the surviving carotid of the ox is roughly proportional to the platelet count of the circulating blood.

2. Bloods with platelet counts far below the normal develop little or no vasoconstrictor property when defibrinated.

3. These findings emphasize the relation of the blood-platelets to the vasoconstrictor property developed in shed blood.

CLINICAL CALORIMETRY

TWENTY-SIXTH PAPER

THE EFFECT OF A SMALL BREAKFAST ON HEAT PRODUCTION*

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AND
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NOTE.—It has been the custom of the Russell Sage Institute of Pathology to publish each year a group of seven or eight papers embodying the work of the previous season. This year practically the whole staff is on active duty in the army or the navy and it has been considered advisable to present three of the papers without waiting for the whole series.

It is hoped at a later date to publish the work on temperature regulation after the intravenous injection of typhoid vaccine, on metabolism in tubercular fever, in erysipelas, in acute and chronic arthritis, and also a summary of the diets that have been used in the metabolism ward. It has been impossible this year to review the literature as fully as has been done in the other papers of this series.

In order to compare the metabolism of different individuals, it has been customary to determine in each case the basal heat production. This means that the subject must be studied in the morning hours, at complete rest, twelve to sixteen hours after the last meal. Hundreds of patients and normal controls have been examined under these standard conditions, which are recognized in almost all the laboratories interested in the gaseous exchanges. Obviously, it would be a mistake to try to alter the standard conditions in any manner that would change the results and render them unfit for comparison with the mass of material already accumulated at the cost of great labor. All who have experimented with large numbers of subjects, however, have been obliged to listen to complaints from those who object to going without breakfast. As a rule, this is not a serious matter, but it is best to have as few complaints as possible from the person who is to be the subject of an experiment which can be profoundly affected by ill humor or nervousness.

Most observers have been afraid to allow their subject any breakfast, because they have believed that the specific dynamic action of the food would increase the metabolism above the basal level. In the

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* From the Russell Sage Institute of Pathology, in Affiliation with the Second Medical Division of Bellevue Hospital.

TABLE 1.—Data of—

Subject. Date, Weight, Surface Area, Ht.-Wt. Formula	Period	End of Period, Time	Carbon Dioxid, Gm.	Oxygen, Gm.	R. Q.	Water, Gm.	Urine N per Hour, Gm.	Indirect Calorimetry, Cal.	Heat Eliminated, Cal.
J. C. F.	Prelim.	11:02	.....	.....	.....	.....	.....	.....	.....
11/10/16	1	12:02	20.6	19.5	0.77	25.4	0.52	62.9	70.4
62.7 Kg.	2	1:02	22.4	19.6	0.83	24.7	0.52	65.4	69.0
1.80 Sq. M.	3	2:07	23.5	19.6	0.87	25.3	0.52	66.1	74.7
J. C. F.	Prelim.	11:06	.....	.....	.....	.....	.....	.....	.....
11/13/16	1	12:06	22.4	19.1	0.85	29.0	0.34	64.3	77.0
62.8 Kg.	2	1:06	26.6	23.2	0.83	28.3	0.34	78.0	80.3
1.80 Sq. M.	Aver.	.....	.....	.....	.....	.....	.....	.....	.....
W. H. O.	Prelim.	11:19	.....	.....	.....	.....	.....	.....	.....
4/7/17	1	12:19	21.7	19.8	0.80	29.3	0.39	65.9	69.6
66.5 Kg.	2	1:19	21.8	19.0	0.84	26.2	0.39	62.6	67.8
1.77 Sq. M.	3	2:19	21.4	20.1	0.77	27.4	0.39	66.5	67.9
	Aver.	.....	.....	.....	.....	.....	.....	.....	.....
W. H. O.	Prelim.	12:07	.....	.....	.....	.....	.....	.....	.....
4/9/17	1	1:07	22.3	19.5	0.83	29.1	0.38	65.4	65.6
66.6 Kg.	2	2:07	21.8	21.2	0.75	28.7	0.38	69.2	67.6
1.77 Sq. M.									
D. P. B.	Prelim.	11:45	.....	.....	.....	.....	.....	.....	.....
11/21/16	1	12:45	23.6	21.6	0.80	27.4	0.55	71.3	74.3
66.2 Kg.	2	1:45	24.6	22.7	0.79	29.9	0.55	75.2	75.1
1.81 Sq. M.	3	2:45	23.6	21.9	0.79	29.3	0.55	72.1	76.4
	Aver.	.....	.....	.....	.....	.....	.....	.....	.....
D. P. B.	Prelim.	11:45	.....	.....	.....	.....	.....	.....	.....
11/22/16	1	12:45	24.3	21.4	0.82	29.4	0.58	71.4	73.9
65.1 Kg.	2	1:45	24.0	22.7	0.77	30.9	0.58	74.4	75.6
1.81 Sq. M.	Aver.	.....	.....	.....	.....	.....	.....	.....	.....
D. P. B.	Prelim.	12:15	.....	.....	.....	.....	.....	.....	.....
11/24/16	1	1:15	26.1	23.1	0.82	32.6	0.57	76.8	74.7
65.3 Kg.	2	2:15	24.9	22.0	0.83	32.8	0.57	73.1	73.3
1.82 Sq. M.	3	3:15	24.3	22.5	0.79	32.3	0.57	74.2	73.6
E. F. D. B.	Prelim.	11:46	.....	.....	.....	.....	.....	.....	.....
12/18/16	1	12:46	25.0	23.3	0.78	38.0	0.44	.....	79.2
73.9 Kg.	2	1:46	23.6	Leak	.....	37.0	0.44	.....	79.4
1.90 Sq. M.	3	2:46	23.6	23.0	0.75	37.0	0.44	.....	78.5
	Aver. 1 and 2	.....	.....	.....	.....	.....	.....	76.2	.....

—Calorimeter Experiments

Direct Calorimetry (Rectal Temp.), Cal.	Rectal Temp., C.	Average Pulse	Work-Adder, Cm.	Non-protein R. Q.	Per Cent. Calories from			Calories per Hour		Remarks
					Protein	Fat	Carbohyd.	Per Kg.	Per Sq. M. (Ht.-Wt.)	
....	36.3	..	..	....	..	..	..	....	....	Standard breakfast, 5:58 a. m.; asleep 20 min.
68.6	36.3	..	10	0.76	22	64	14	1.02	35.5	Very quiet
65.0	36.2	56	7	0.84	21	42	37	1.04	36.3	Very quiet
76.8	36.3	..	..	0.89	23	28	49	0.97	33.9	Very quiet
....	36.7	60	..	....	..	..	..	....	....	Basal
61.5	36.4	..	15	0.80	..	..	..	1.02	35.7	Fairly quiet
80.3	36.4	68	24	0.84	..	..	..	1.24	43.3	Restless; excluded from basal average
....	....	..	..	....	13	45	42			
....	36.5	..	..	....	..	..	..	....	....	Standard breakfast, 6:35 a. m.
69.3	36.5	..	..	0.80	16	59	25	0.99	....	Fairly quiet; slept 35 min.
72.4	36.6	..	..	0.84	16	45	38	0.96	....	Quiet
67.5	36.6	..	..	0.77	15	66	19	1.00	....	Very quiet
....	....	..	..	....	..	..	..	....	36.1	
....	36.5	..	..	....	..	..	..	....	....	Basal
64.8	36.5	..	10	0.84	15	46	39	0.98	37.0	Very quiet
64.0	36.4	..	9	0.74	15	52	33	1.04	39.1	Very quiet
....	36.8	70	..	....	..	..	..	....	....	Standard breakfast, 6:45 a. m.
54.5	36.5	..	5	0.80	20	55	25	1.09	....	Very quiet
75.3	36.5	64	6	0.78	19	60	21	1.15	....	Very quiet
82.6	36.6	64	7	0.78	20	59	21	1.10	....	Very quiet
....	....	..	..	....	..	..	..	....	40.2	
....	36.9	..	..	....	..	..	..	....	....	Basal
67.1	36.7	69	6	0.83	21	46	33	1.10	....	Very quiet
75.8	36.7	64	13	0.75	21	65	14	1.14	....	Very quiet
....	....	..	..	....	..	..	..	....	40.0	
....	36.7	75	..	....	..	..	..	....	....	Standard breakfast, 11:56 a.m. 12:01 p.m.
59.7	36.5	70	8	0.83	20	47	33	1.13	42.4	Asleep 15 min.; very quiet
67.0	36.7	..	13	0.83	20	47	33	1.12	40.4	Very quiet
71.1	36.7	67	11	0.78	20	59	21	1.14	42.0	Very quiet
....	37.0	..	..	....	..	..	..	....	....	Basal
75.7	36.9	60	13	....	..	..	..	....	....	Very quiet
71.6	36.8	..	12	....	..	..	..	....	....	Leak in oxygen; hour lost
81.1	36.8	60	15							
....	....	..	..	0.76	15	71	14	1.08	40.1	

TABLE 1.—Data of Calorimeter—

Subject, Date, Weight, Surface Area, Ht.-Wt. Formula	Period	End of Period, Time	Carbon Dioxid, Gm.	Oxygen, Gm.	R. Q.	Water, Gm.	Urine N per Hour, Gm.	Indirect Calorimetry, Cal.	Heat Eliminated, Cal.
E. F. D. B.	Prelim.	11:37	.....	.....	.....	.....	.....	.....	.....
12/30/16 74.3 Kg. 1.90 Sq. M.	1	12:37	26.9	24.0	0.82	39.1	0.47	.....	81.0
	2	1:37	26.3	22.5	0.85	37.9	0.47	.....	79.9
	3	2:37	24.6	23.6	0.76	38.1	0.47	.....	79.5
	Aver.	.....	.....	.....	.....	.....	.....	77.8	.....
L. O'B.	Prelim.	11:56	.....	.....	.....	.....	.....	.....	.....
4/17/17 45.8 Kg. 1.42 Sq. M.	1	12:56	17.2	15.3	0.82	29.2	0.31	.....	57.1
	2	1:56	17.9	16.2	0.80	25.3	0.31	.....	58.7
	Aver.	.....	17.5	15.8	0.81	27.2	.....	52.5	55.4
	3	2:20	.....	.....	.....	.....	.....	.....	.....
	4	3:20	20.2	17.3	0.85	27.4	0.31	58.3	55.2
	5	4:20	19.5	16.4	0.87	25.9	0.31	55.4	54.3
	Aver.	.....	19.9	16.9	0.86	26.7	0.31	56.9	54.8

present paper it is possible to show that the increase caused by a small standard breakfast is so transient that it can safely be ignored two hours after the meal is taken. As a result of the experiments here reported the custom has recently been adopted of allowing almost all the patients and normal controls to eat a small breakfast five or six hours before the observation begins. In consequence of this change the subjects have been noticeably better humored and stand quite easily observations lasting until late in the afternoon. Previous to the use of the small breakfast it was often necessary to end the calorimeter experiments about 2 o'clock in the afternoon, because the patients felt weak from lack of food.

The subject of the specific dynamic action of food has been discussed in Papers 4, 7 and 21 of this series,[1] and it is fully reviewed by Graham Lusk.[2] The increase in heat production following a large protein meal may amount to 46 per cent. in man, and in the dog some

1. Gephart, Frank C., and Du Bois, Eugene F.: Clinical Calorimetry, Paper 4, The Determination of the Basal Metabolism of Normal Men and the Effect of Food, The Archives Int. Med., 1915, **15,** 835. Coleman, Warren and Du Bois, E. F.: Clinical Calorimetry, Paper 7, Calorimetric Observations on the Metabolism of Typhoid Patients With and Without Food, *Ibid.*, 1915, **15,** 887. Aub, J. C., and Du Bois, E. F.: Clinical Calorimetry, Paper 21, The Basal Metabolism of Dwarfs and Legless Men with Observations on the Specific Dynamic Action of Protein, *Ibid.*, 1917, **17,** 840.

2. Lusk, Graham: The Elements of the Science of Nutrition, Ed. 3, Philadelphia, 1917.

—EXPERIMENTS—(*Continued*)

Direct Calorimetry (Rectal Temp.), Cal.	Rectal Temp., C.	Average Pulse	Work-Adder, Cm.	Non-protein R. Q.	Per Cent. Calories from			Calories per Hour		Remarks
					Protein	Fat	Carbohyd.	Per Kg.	Per Sq. M. (Ht.-Wt.)	
....	36.9	..	..	....	..	..	..	....	....	Standard breakfast, 11:18–11:23 a. m.
76.9	36.9	58	10	....	..	..	..	....	....	Very quiet
80.1	36.9	58	10	....	..	..	..	....	....	Very quiet
75.5	36.8	58	12	....	..	..	..	....	....	Very quiet
....	....	..	..	0.81	16	55	29	1.05	41.0	
....	37.0	..	..	....	..	..	..	....	....	Basal
54.5	37.0	..	7	....	..	..	..	....	....	Very quiet
51.9	36.9	55	7	....	..	..	..	1.15	37.0	Very quiet
53.2	36.9	..	..	0.81	16	55	29			
....	37.0	..	..	....	..	..	..	....	....	Standard breakfast, 1:58–2:06 p. m.
52.9	37.0	..	6	0.85	14	43	43	1.27	41.1	Very quiet
53.6	37.0	91	1	0.88	15	35	50	1.21	39.0	Very quiet
52.3	37.0	..	..	0.87	15	30	46	....	40.1	

effect has been apparent for as long as twenty hours. With carbohydrate and fat the effect is less striking, but a rise of 20 per cent. is not uncommon. Most experimenters have confined their attention to the results after large meals. In order to study small meals the technic is difficult, since it is necessary to detect changes of 2 per cent. in the heat production.

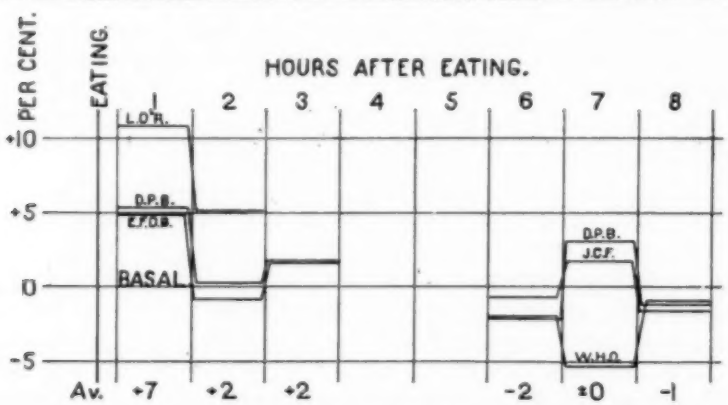
For this reason the subjects for the following experiments were chosen with great care. All of them were laboratory workers thoroughly familiar with the calorimeter, and they appreciated the importance of remaining quiet. Four of the subjects were men; one was a woman. Although their weights varied between 45 and 74 kg., they were all given exactly the same amount of food. The standard breakfast selected consisted of a slice of bread (30 gm.) with 8 gm. butter and a cup (200 c.c.) of caffein-free coffee (Kaffee Hag) containing 10 gm. cane sugar and 60 c.c. milk. This contains 4.7 gm. protein, 9.0 gm. fat and 28.9 gm. carbohydrate, a total of 222 calories, using the following figures as the average composition of the foods taken:

	Protein, Per Cent.	Fat, Per Cent.	Carbohydrate, Per Cent.
Bread	9.8	0.3	53.5
Butter	...	85.0	....
Sugar	...	...	100.0
Milk	3.1	3.5	4.7

This is very similar to the small breakfast allowed the Boy Scouts in Papers 12[3] and 27[4] of this series. In some of the experiments the subject took the food while in the calorimeter after two basal periods; in some the meal was taken at home shortly after rising.

TABLE 2.—STANDARD BREAKFAST—SUMMARY TABLE

Subject	Sex	Age	Wt., Kg.	Ht., Cm.	Total Calories		Cal. per Sq. M. per Hr.: Basal	Per Cent. Relation to Av. Normal	Remarks
					Direct	Indirect			
J. C. F.	♂	23	62.7	180	210.4	195.4	35.2	....	5 hours after standard breakfast
J. C. F.	♂	23	62.8	180	141.8	142.3	39.5	−10	No breakfast
W. H. O.	♂	29	66.5	170	200.2	196.1	36.1	....	5 hours after standard breakfast
W. H. O.	♂	29	66.6	170	128.7	134.6	38.0	−4.5	No breakfast
D. P. B.	♂	27	65.3	177	212.3	218.6	40.2	....	5 hours after standard breakfast
D. P. B.	♂	27	65.1	177	142.9	145.8	40.0	+1	No breakfast
D. P. B.	♂	27	65.3	177	217.8	224.1	41.2	....	15 minutes after standard breakfast
E. F. D. B.	♂	34	73.9	179	228.5	*	40.1	+1	No breakfast
E. F. D. B.	♂	34	73.5	179	232.4	233.3	41.0	....	15 minutes after standard breakfast
L. O'R.	♀	25?	45.8	156	106.5	104.8	37.0	00	No breakfast
L. O'R.	♀	..	45.8	156	106.6	113.7	40.1	....	15 minutes after standard breakfast



Graphic demonstration of change in the level of metabolism following the ingestion of the standard breakfast. The lines are drawn for each experiment according to the percentage deviation from the individual's own basal metabolism (Line O).

3. Du Bois, E. F.: Clinical Calorimetry, Paper 12, The Metabolism of Boys Twelve and Fourteen Years Old Compared with the Metabolism at Other Ages, THE ARCHIVES INT. MED., 1916, **17**, 887.

4. Immediately following this paper.

DISCUSSION OF RESULTS

Although the series of experiments is not as large as might be desired, one point is clearly demonstrated, and that is the small and transient character of the rise in metabolism following a small breakfast. During the first hour after the ingestion of the food the increase was 12 per cent. in one case and 5 and 6 per cent. in the others. In the second hour the average was 2 per cent.; in the third hour 2 per cent., and in the sixth, seventh and eighth hours the figures were actually 0.2 per cent. lower than in the fasting, basal hours. It is doubtful if a difference of 2 per cent. is of any significance, because in basal experiments the results in hourly periods often show greater divergence. No experiments were made more than eight hours after the standard breakfast, but there is no reason to suppose that there would be a delayed rise in metabolism after a small meal. No such phenomenon has been seen in experiments on the specific dynamic action after large meals.

The standard breakfast contained approximately 222 calories, enough to supply the basal energy requirement of the subjects three to four hours. When the breakfast was taken at home and the subjects traveled to the laboratory their metabolism was, perhaps, double the basal figures. In either case the food or its equivalent was all oxidized within a few hours, and it is perfectly natural that there should be little or no specific dynamic action after the complete utilization of the food. It is obvious that we can determine the level of the basal metabolism within six hours of the taking of the standard breakfast described above. The experimental conditions are made much easier for the subject, and it is believed that better results will be obtained. Some may object to using the term "basal" for results obtained unless the subject is "breakfastless" or "nüchtern," since the original conditions demanded that a period of twelve to sixteen hours must have elapsed since the last meal. On the other hand, no attention was previously paid to the size of that meal, and it is not impossible that there may be some specific dynamic action fourteen hours after a heavy protein dinner.

This brings us to the economic question as to whether it is better to take two or three large meals a day or to divide the food into five or six small meals. Judging from the results of the present experiments, the frequent meals, just supplying the energy as it is required, would diminish the specific dynamic action and result in a saving equivalent to, perhaps, 5 or 10 per cent. of the basal metabolism, or about 200 calories a day. Practically, it would be of little importance, since some of the specific dynamic action of the food is utilized in the increased metabolism of work. In this connection it is well to recall

that Karl Thomas found that he could establish a lower nitrogen minimum if he took his protein food in nine small doses instead of taking it in three meals. On the other hand, we must remember the waste of time in taking frequent meals and the tendency to overeat.

SUMMARY AND CONCLUSIONS

Ten experiments were made on five subjects to determine the extent of the rise in metabolism following a small meal. The standard breakfast used in all observations consisted of 30 gm. bread, 8 gm. butter, 10 gm. sugar, and 60 c.c. milk, amounting to 4.7 gm. protein, 9.0 gm. fat, and 28.9 gm. carbohydrate, or 222 calories. In the first hour following the ingestion of this the heat production increased on an average 7 per cent.; in the second hour, 2 per cent.; in the third hour, 2 per cent. In the sixth, seventh and eighth hours the metabolism was slightly lower than before the breakfast. It is evident that when this small amount of food is taken for breakfast it is only during the first hour that the absorption of food could have been in sufficient quantity to produce the condition of a "metabolism of plethora."

477 First Avenue.

CLINICAL CALORIMETRY

TWENTY-SEVENTH PAPER

METABOLISM OF BOYS TWELVE AND FOURTEEN YEARS OLD*

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The question of the effect of age on heat production was discussed in Paper 12 of this series[1] and the curve was drawn showing that metabolism is relatively high in childhood, falls rapidly during adolescence, then decreases slowly during the rest of life. In Paper 19[2] it was shown that the curve needed slight alteration in extreme old age.

At the time the Boy Scouts were first studied, in March and April, 1915, it was hoped that the same boys could be studied every year in order to plot a curve for each individual. Unfortunately, this was impossible in 1916, and it was not until March and April, 1917, that the boys could be observed again in the calorimeter. As will be seen from the accompanying charts, the changes in metabolism of the individual boys correspond so closely with the chart made two years ago that it is not necessary to alter the curve or change the conclusions made at that time.

All of the boys examined in 1915 were studied in 1917 under experimental conditions which were practically unchanged. With the exception of J. D. D. B. they came in from nearby suburbs on the morning of the observation. All but one of the boys took a breakfast consisting of a glass of milk and a slice of toast, with butter, between the hours of half past 6 and half past 7 in the morning.

The histories of the boys during the last two years are given below:

F. R. S., aged 14 years, 11 months, has been perfectly well during the last two years except for earache with purulent discharge in 1916. He has grown 14 cm. His voice has not yet changed; sex organs of moderate size; pubic hair fairly

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1. Du Bois, E. F.: Clinical Calorimetry, Paper 12. The Metabolism of Boys 12 and 13 Years Old Compared with the Metabolism at Other Ages. THE ARCHIVES INT. MED., 1917, **17**, 887.

2. Aub, J. C., and Du Bois, E. F.: Clinical Calorimetry, Paper 19. The Basal Metabolism of Old Men. THE ARCHIVES INT. MED., 1917, **19**, 823.

TABLE 1.—

Subject, Date, Weight, Surface Area, Linear Formula	Period	End of Period	Carbon Dioxid, Gm.	Oxygen, Gm.	R. Q.	Water, Gm.	Urine N per Hour, Gm.	Indirect Calorimetry, Cal.	Heat Eliminated, Cal.
F. R. S.	Prelim.	12:15	.....	.....	.....	.....	.....	.....	.....
4/5/17									
42.61 Kg.	1	1:15	23.01	20.29	0.825	38.32	0.470	67.66	68.39
1.37 Sq. M.	2	2:15	22.68	20.46	0.840	36.70	0.470	68.59	68.18
	Aver.	.....	22.82	20.38	0.832	37.61	0.470	68.13	68.28
J. D. D. B.	Prelim.	11:46	.....	.....	.....	.....	.....	.....	.....
3/30/17									
45.53 Kg.	1	12:46	22.56	21.84	0.751	29.69	0.501	.....	75.85
1.52 Sq. M.	2	1:46	23.22	21.67	0.846	37.42	0.501	.....	76.77
	Aver.	.....	22.89	21.75	0.798	33.55	0.501	72.2	76.31
Raymond M.	Prelim.	12:20	.....	.....	.....	.....	.....	.....	.....
3/27/17									
49.09 Kg.	1	1:20	18.59	15.78	0.856	26.43	0.441	.....	55.98
1.24 Sq. M.	2	2:20	19.43	16.67	0.848	24.95	0.441	.....	56.51
	Aver.	.....	19.01	16.22	0.853	25.69	.....	54.4	56.24
Reg. F.	Prelim.	12:20	.....	.....	.....	.....	.....	.....	.....
3/28/17									
48.55 Kg.	1	1:20	18.42	17.77	0.754	27.27	0.483	.....	58.07
1.52 Sq. M.	2	2:20	21.49	17.82	0.877	28.68	0.483	.....	60.64
	Aver.	.....	19.95	17.79	0.815	27.97	0.483	59.1	59.35
Harry B.	Prelim.	2:09	.....	.....	.....	.....	.....	.....	.....
3/29/17									
49.09 Kg.	1	2:39	10.84	9.58	0.822	17.59	0.423	.....	34.32
1.51 Sq. M.	2	3:39	21.50	19.94	0.784	38.45	0.423	.....	70.86
	Aver.	.....	16.17	14.76	0.803	28.02	0.423	65.3	52.59
Henry K.	Prelim.	12:22	.....	.....	.....	.....	.....	.....	.....
4/2/17									
49.29 Kg.	1	1:22	22.23	19.82	0.816	27.88	0.330	.....	61.83
1.54 Sq. M.	2	2:22	22.60	19.56	0.824	30.20	0.330	.....	67.16
	Aver.	.....	22.41	19.80	0.820	29.04	0.330	66.6	64.49
Arthur A.	Prelim.	11:18	.....	.....	.....	.....	.....	.....	.....
4/3/17									
39.46 Kg.	1	12:18	20.85	18.67	0.812	26.18	0.367	.....	65.73
1.37 Sq. M.	2	1:18	20.79	18.26	0.828	24.97	0.367	.....	64.87
	Aver.	.....	20.82	18.46	0.825	25.57	0.367	61.8	65.30
Leslie B.	Prelim.	11:23	.....	.....	.....	.....	.....	.....	.....
3/29/17									
34.20 Kg.	1	11:53	9.67	8.64	0.814	13.50	0.513	.....	29.79
1.20 Sq. M.	2	12:53	18.90	15.00	0.916	26.53	0.513	.....	55.62
	Aver.	.....	14.29	11.82	0.865	20.01	0.513	53.1	42.70

—Calorimeter Data

Direct Calorimetry (Rectal Temp.), Cal.	Rectal Temp., C.	Average Pulse	Work-Adder, Cm.	Non-protein R. Q.	Per Cent. Calories from			Calories per Hour		Remarks
					Protein	Fat	Carbohyd.	Per Kg.	Per Sq. M. (Linear)	
.....	37.09	..	..	.....	..	..	..	.....	.....	Basal
62.31	36.93	76	13	.....	..	..	..	.....	.....	Quiet; reading 50 min.
69.29	36.97	..	16	.....	..	..	..	.....	.....	Quiet; reading 20 min.
65.80	36.99	..	14	0.84	18	45	37	1.60	49.7	
.....	37.33	77	..	.....	..	..	..	.....	.....	Basal; not very satisfactory; excluded from averages and chart
61.82	36.97	72	10							
84.01	37.17	84	16							Third period started; removed from calorimeter because of pain in leg
72.91	37.16	77	13	0.80	18	57	25	1.59	47.5	
.....	37.12	68	..	.....	..	..	..	.....	.....	Basal
52.94	47.08	..	3	.....	..	..	..	.....	.....	Motionless
55.10	36.99	78	3	.....	..	..	..	.....	.....	Motionless
54.02	37.05	73	3	0.87	21	36	43	1.11	43.9	
.....	36.99	..	..	.....	..	..	..	.....	.....	Basal
50.48	36.81	..	8	.....	..	..	..	.....	.....	Quiet; asleep 20 min.
56.28	36.71	63	9	.....	..	..	..	.....	.....	Quiet
53.38	36.84	..	8	0.82	22	48	30	1.22	39.1	
.....	37.33	..	..	.....	..	..	..	.....	.....	Basal
28.40	37.19	75	4	.....	..	..	..	.....	.....	Reading 5 min.; very quiet
59.44	36.92	..	3	.....	..	..	..	.....	.....	Very quiet
43.92	37.15	..	3	0.80	17	58	25	1.33	43.2	
.....	36.99	71	..	.....	..	..	..	.....	.....	Basal
58.74	36.80	75	5	.....	..	..	..	.....	.....	Very quiet; reading 8 min.
69.71	36.87	72	2	.....	..	..	..	.....	.....	Very quiet; reading 60 min.
61.72	36.80	73	..	0.82	13	63	24	1.35	43.3	
.....	36.98	..	..	.....	..	..	..	.....	.....	Basal
56.91	36.72	..	8	.....	..	..	..	.....	.....	Very quiet; reading 50 min.
63.36	36.68	82	11	.....	..	..	..	.....	.....	Very quiet; reading 50 min.
60.13	36.79	..	9	0.83	16	50	34	1.57	45.1	
.....	37.19	..	..	.....	..	..	..	.....	.....	Basal
26.75	37.09	..	5	.....	..	..	..	.....	.....	Very quiet
50.18	36.91	65	15	.....	..	..	..	.....	.....	Somewhat restless
38.46	37.06	..	10	0.91	26	23	51	1.55	44.3	

TABLE 2.—Showing the Relation Between the Basal Metabolism and the Increase—

Name	First Experiment		Second Experiment		Weight in Kg.			Height in Cm.			Calories per Hour		
	Age, Years	Signs of Puberty	Age, Years	Signs of Puberty	First Exper.	Second Exper.	Increase in per Cent.	First Exper.	Second Exper.	Increase in per Cent.	First Exper.	Second Exper.	Change in per Cent.
L. B.	13 3/12	±	14 3/12	++	28.5	34.2	20	141	151	7	54.1	58.1	— 2
R. M.	12 6/12	0	14 6/12	±	30.4	49.1	61	141	149	6	59.0	54.4	— 8
R. F.	12 8/12	+	14 8/12	+++	35.4	48.6	36	148	166	12	61.8	59.1	— 4
F. R. S.	12 10/12	0	15	+	32.1	42.6	33	142	156	10	58.6	68.1	+18
A. A.	13 8/12	0	15 8/12	+	30.6	39.5	29	147	160	9	56.1	61.8	+10
H. B.	13 10/12	++	15 10/12	+++	36.6	49.1	34	146	162	11	58.4	65.3	+12
H. K.	13 11/12	±	15 11/12	++	36.0	49.3	37	148	166	12	60.0	66.6	+11
Aver.	.....	.....	.....	.....	32.8	48.1	46	145	159	10	58.3	61.2	+ 5

abundant. During the experiment he was quiet. This boy took a somewhat larger breakfast than the others. At 7 a. m. he had two slices of toast and a cup of coffee with sugar and cream.

J. D. D. B., aged 14 years, 2 months, has been perfectly well during the last two years and has grown 16 cm. His voice is just beginning to change; genitalia about one-half adult development; pubic hair about one-quarter present; scant hair in axillae; slight down on lip.

The experiment of this boy was not satisfactory. He tried so hard to keep quiet that he developed a severe cramp in the leg. He was in great pain, especially during the second period. This probably accounts for his relatively high metabolism. The results are not used in the averages and are not shown on the graphic chart.

Raymond M., aged 14 years, 7 months, has grown only 8 cm. His health has been good. His voice has not yet changed; genitalia are just beginning to develop; pubic hair scant; a few axillary hairs are present; no down on lip. During the experiment he was motionless.

Reginald F., aged 14 years, 8 months, has grown 18 cm. Two weeks before the observation he had a mild attack of cramps in the abdomen; otherwise well. Three months prior to the observation he had to stop singing in a choir on account of change in voice. Genitalia are adult in type; pubic hair is abundant; and there is a slight growth of down on the lip. During the observation he was quiet.

Harry B., aged 15 years, 10 months, has grown 15.5 cm. His health has been good. The voice and genitalia are of adult type; pubic and axillary hair abundant; slight down on lip. During the observation he was quiet.

Henry K., aged 15 years, 11 months, has grown 18 cm. His health has been good. His voice is changing; genitalia are about three-quarters adult development; pubic hair all present; axillary hair about one-half present; slight down on lip. He was very quiet during the experiment.

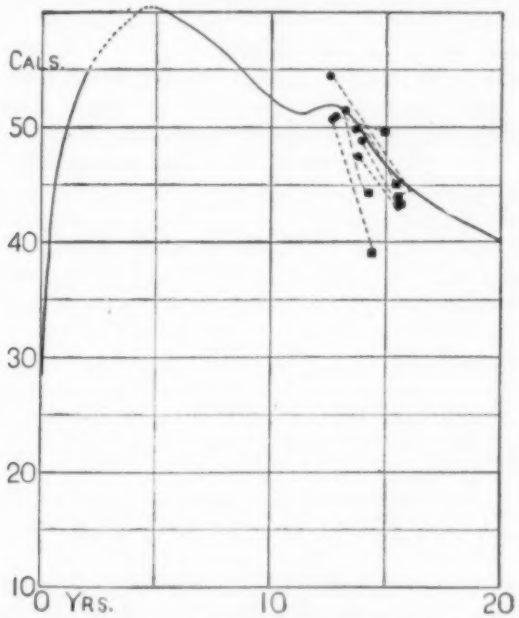
Arthur A., aged 15 years, 8 months, has grown 13 cm. Although small and slight he has won a medal as the best all-round Boy Scout in his troop. During the last two years he has been perfectly well. The voice has not yet changed; genitalia are about one-quarter way toward adult development; no hair in axillae or on lip. Both mammary glands are palpable, about 1 cm. in diameter; they are not tender. He was very quiet during the observation.

Leslie B., aged 14 years, 3 months. During the last two years he has gained 10.5 cm. and has been perfectly well except for a broken finger and a dog bite.

—IN HEIGHT AND WEIGHT AND OTHER FACTORS OF BOYS AT THE TIME OF PUBERTY

Calories per Sq. M. Surface per Hour			Calories per Kg. per Hour		Average R. Q.		Circumference of Thorax		Surface Area (Linear Formula)		Average Pulse	
First Exper.	Second Exper.	Decrease in per Cent.	First Exper.	Second Exper.	First Exper.	Second Exper.	First Exper.	Second Exper.	First Exper.	Second Exper.	First Exper.	Second Exper.
							Cm.	Cm.	Sq. M.	Sq. M.		
51.6	44.3	—14	1.90	1.55	0.82	0.87	61.8	67.5	1.05	1.20	88	65
54.4	43.9	—19	1.94	1.11	0.81	0.85	65.8	68.5	1.08	1.24	85	73
50.7	39.1	—23	1.75	1.22	0.94	0.82	68.2	76.6	1.22	1.52	79	63
51.0	49.7	— 3	1.79	1.60	0.83	0.83	66.2	73.0	1.12	1.37	80	76
49.9	45.1	—10	1.84	1.57	0.78	0.82	65.4	71.0	1.13	1.37	81	82
47.4	43.2	— 9	1.60	1.33	0.86	0.80	71.4	80.2	1.23	1.51	88	75
48.8	43.3	—11	1.66	1.35	0.86	0.82	67.7	77.3	1.22	1.54	87	73
50.6	44.1	—13										

His voice is beginning to change; genitalia about one-half developed; pubic hair about one-half present; scant hair in axillae; no down on lip. During the first hour of observation he was very quiet; during the second, somewhat restless.



Reproduction of original age curve showing relationship of findings to the line drawn in 1915. Each round dot represents the calories per square meter per hour for the boy in 1915. A dash line connects it with the square which represents the result obtained in 1917.

DISCUSSION OF RESULTS

The results are shown graphically in the chart. All of the individuals show a marked decrease in metabolism. In the case of F. R. S. this is not as striking as in the others. Although the majority of the boys come a little below the line that was drawn two years ago, the change is not great enough to make it advisable to draw a new curve. The important fact remains that there is a rapid fall in metabolism during adolescence. The average decrease for the seven satisfactory cases studied is 13 per cent. Their average metabolism at the ages of 14 and 15 years is 44.1 calories per square meter per hour, which is 11 per cent. above the average for adult men between the ages of 20 and 40 years.

In the three youngest boys the metabolism during the twelfth year was actually greater in calories produced than during the fourteenth year, although the boys showed a gain in weight of between 35 and 50 per cent.

477 First Avenue.

CLINICAL CALORIMETRY

TWENTY-EIGHTH PAPER

THE METABOLISM IN MALARIAL FEVER*

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NEW YORK

I. INTRODUCTION

When the respiration calorimeter of the Russell Sage Institute of Pathology was originally planned one of the main problems in view was the study of the rise and fall of the body temperature in fever. For the first time it became possible to measure in the clinic practically all the factors which would tell the story as to what was taking place within the body. The preliminary work that was done in typhoid fever, with its comparatively slow changes in body temperature, demonstrated the extraordinary difficulty of the technic. One case of malaria studied that same year gave warning of the troubles to be encountered when the temperature fluctuations were rapid. It was for this reason that the main study of malaria was postponed until the calorimeter staff had been drilled four years and the technic and apparatus so improved that the problem seemed capable of solution.

The calorimeter is a bulky apparatus and does its best work when the metabolic condition of the patient remains unchanged from hour to hour. With the onset of the malarial chill there is an abrupt change in the patient's metabolism and the calorimeter must be rapidly adjusted to meet the new conditions. Again, at the termination of the chill there is another change, and at the outbreak of sweat a new set of conditions is encountered. In previous years it had been the custom to make the periods of observation one hour in length, and even these periods kept the staff busy. This year it was found possible to obtain satisfactory results in periods as short as half an hour, and in some instances twenty-two minutes. In this manner one could study different portions of the malarial chill, whereas the one-hour periods would necessarily have included two or three different phases of the paroxysm.

The problems were to determine the influence of fever on the level of heat production, and to see whether the latter is proportional to the

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* From the Russell Sage Institute of Pathology, in affiliation with the Second Medical Division of Bellevue Hospital.

height of the temperature, or if the height of the temperature depends on the level of the metabolism. Even more important was the study of the mechanism by which the body raises or lowers its temperature. Does the temperature rise because the heat production has been increased or because the heat elimination has been diminished, or from a combination of these two causes? Does the body temperature fall because the heat production is diminished, or because the heat elimination is increased, or, again, from a combination of the two? Still another problem is the measurement of the average temperature change within the body. Hitherto our knowledge of the temperature change has been dependent on thermometers in the mouth, rectum or axillae. Another interesting problem was the comparison of the temperature regulation during malarial chill and the chills following the intravenous injection of a foreign protein. Finally, there remained the interesting question as to the character of the foodstuffs oxidized during a chill.

II. THE MECHANISM OF THE RISE AND FALL OF TEMPERATURE

Any change in body temperature is due to a disturbed relation between two factors, heat production and heat elimination. Heat production is the result of the combustion of foodstuffs within the body. If the body is to remain at a constant temperature, the heat thus produced must be eliminated. This is accomplished chiefly by two avenues of heat loss: first, radiation and conduction of heat to the surrounding air, and second, the vaporization of water eliminated from the skin and lungs. Every gram of water which is vaporized at 23 C. absorbs 0.584 of a calory. Ordinarily in afebrile conditions the heat lost in the vaporization of water constitutes about one quarter of the total heat eliminated. A slight amount of heat is lost in the urine and feces and in warming food and drink which is introduced into the body at a low temperature. The other three quarters, however, is lost chiefly by radiation and conduction. In afebrile conditions the heat production equals the heat elimination, thus keeping the body temperature at a constant level.

We must decide whether fluctuation in temperature is due to a change in heat production or in heat elimination. A theoretical consideration of the problem would indicate that both must be affected.

To raise 1 kg. of water 1 degree C. requires one calory of heat. To raise 1 kg. of body tissue 1 degree C. requires only 0.83 calory. In other words, the specific heat of the body is said to be 0.83. If, therefore, the temperature of a man weighing 70 kg. should rise 1 degree C. within an hour, 70×0.83 or 58.1 calories would necessarily be stored in that man's body. The ordinary heat production of the man at rest would be about 70 calories per hour. If there were no increase in heat

production only 11.9 calories, or about a sixth of the usual amount of heat, would be eliminated. This does not seem probable.

On the other hand, it is a well known fact that heat production is enormously increased during severe exercise, such as mountain climbing or running, or during shivering after exposure to cold. With this increase in metabolism, however, there is no marked rise in temperature. After the ingestion of large amounts of protein food the heat production may be increased 50 per cent. or more, and yet there is no rise in body temperature. The explanation is that in these conditions the heat elimination is increased to compensate for and to equal the extra heat production. Thus, it would appear that there is in fever some disturbance in the power of the body to eliminate heat.

The problem, therefore, is to determine how much and in what manner each of these factors acts to produce changes in body temperature.

Much careful work has been done on this problem with quite conflicting results. Traube,[1] as long ago as 1863, studied the question of the mechanism of fever. He attributed the cause of fever to a sudden contraction of the peripheral blood vessels which prevented the proper distribution of blood to the surface of the body and thus interfered with a normal heat loss. Those who followed Traube's teaching believed that the cause of fever was not an increase in heat production, but a decrease in heat elimination. Senator[2] believed that the rise in body temperature took place in consequence of an abnormally high heat production with a heat elimination not correspondingly high. Senator thus assumed an increase in the production of heat which Traube did not find. Leyden[3] found a considerable increase in heat production.

All of the earlier workers were handicapped by imperfect and incomplete apparatus. The problem could not be finally settled except by an instrument which very accurately measured heat production and respiratory exchange simultaneously in short periods. This has been possible for the first time with the calorimeter of the Russell Sage Institute of Pathology. Using this instrument in their observations on typhoid fever, Coleman and Du Bois[4] found that an increase in heat production accompanied a rising body temperature in six out of seven cases observed. The heat elimination was not equal to the heat production, but rose to meet the higher level of metabolism. One observa-

1. Traube: Allg. med. Centr.-Ztg., 1863, **32,** 410, 426 and 810.
2. Senator: Allg. med. Centr.-Ztg., 1868, **37,** 926; Untersuchungen über die fieberhaften Prozesse, Berlin, 1873.
3. Leyden: Deutsch. Arch. f. klin. Med., 1870, **7,** 536.
4. Coleman, W., and Du Bois, E. F.: Clinical Calorimetry, Paper 7, Calorimetric Observations on the Metabolism of Typhoid Patients With and Without Food, THE ARCHIVES INT. MED., 1915, **15,** 887.

tion showed a decrease in both heat production and heat elimination. These observers also found that when the body temperature is constant at high fever the heat production and heat elimination are equal to each other. When the body temperature falls the heat elimination rises above the heat production, while the amount of the latter may or may not fall.

The fluctuation of temperature in the typhoid cases was not striking, the maximum change being only 0.7 C. The present work deals with more rapid and with greater changes and should, therefore, yield more decisive results.

III. REVIEW OF LITERATURE

The first of the conditions to be considered is malarial fever. Some very important work has previously been done on the metabolism of this disease.

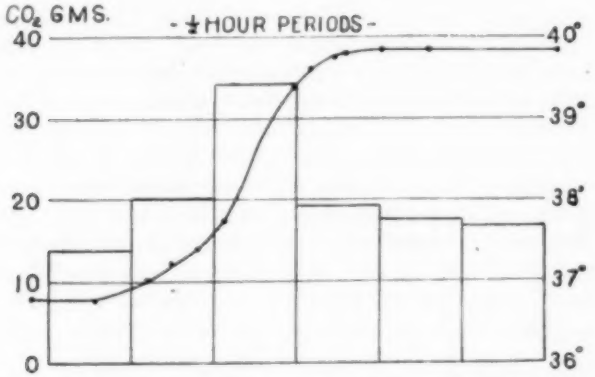

Fig 1.—Curved line shows the body temperature and the columns the carbon dioxid elimination of Liebermeister's patient during a malarial paroxysm.

In 1870 Liebermeister[5] studied the carbon dioxid output of two patients before, during and after a malarial chill. He considered that the carbon dioxid expired accurately indicated the heat production. How correct this supposition was can be judged by comparison of the chart given by Liebermeister with our record of the patient George S. (page 633). Liebermeister demonstrated that the chill is accompanied by a great increase in heat production. Following the chill the heat production drops to a level not far above that observed before the paroxysm began. He believed that the level of heat production is only slightly dependent on the absolute height of the body temperature. A glance at the accompanying chart (Fig. 1) will show that this is true. During the rapid rise of temperature accompanying the chill there is a great increase in the production of carbon dioxid.

5. Liebermeister: Deutsch. Arch. f. klin. Med., 1871, **7**, 153.

After the chill the temperature continues to rise although the heat production suddenly drops nearly to its former level. Liebermeister very aptly compares this to the action of the sun on the temperature of the air on the earth's surface. The sun, which would correspond to the heat production, exerts its maximum heat at midday. During this period the temperature of the air rises rapidly. After noon, although the heat of the sun becomes less intense, the temperature of the air continues to rise for a considerable period and remains high even when the sun is set.

The first calorimetric observations of a patient with malaria were made in 1892 by Isaac Ott[6] of Philadelphia, who used an instrument which required a plus correction of 16 per cent. He found during a chill a great increase in heat production, with a considerable decrease in heat elimination. He also confirmed Liebermeister's finding that following the chill the heat production falls, although the temperature still remains high. During the fall in temperature he found a great increase in heat elimination and in the elimination of water, without marked change in the heat production.

Probably the best known work on the rise and fall of temperature in malaria was done by Likhatscheff and Avroroff[7] in 1902. As excellent abstracts of this most important research have been made by Ott[6] and by A. I. Ringer,[8] it will not be necessary to review the details of their methods. They used a large Paschutin calorimeter with which they measured both the heat production by the direct method and the carbon dioxid output. Their results are most interesting and important. They found that an increase in heat production occurred during a rise in temperature; an increased heat elimination and output of water during the fall. The output of carbon dioxid increased greatly during the rise in temperature but lagged behind the maximum heat production. They concluded that a rise in body temperature depends on an increased heat production.

IV. METHODS OF INVESTIGATION

In the following experiments the calorimeter of the Russell Sage Institute was employed. This has been described in Paper 2 of this series.[9] The heat production was measured by both direct and indirect

6. Ott, Isaac: Fever. Its Thermotaxis and Metabolism, New York, 1914.

7. Likhatscheff and Avroroff: Investigations of Gaseous and Heat Exchange in Fevers. Report of the Imperial Military Academy, St. Petersburg, 1902, **5,** Parts 3 and 4. We are indebted to Dr. F. G. Benedict of the Carnegie Nutrition Laboratory for permission to consult his translation of this work.

8. Ringer, A. I.: Physiology and Pathology of Fever, Am. Jour. Med. Sc., 1911, **142,** 485.

9. Riche, J. A., and Soderstrom, G. F.: Clinical Calorimetry, Paper 2. The Respiration Calorimeter of the Russell Sage Institute of Pathology in Bellevue Hospital, THE ARCHIVES INT. MED., 1915, **15,** 805.

methods, the factors of which have nothing in common. The direct method measures the heat lost in radiation and conduction directly. The vaporized water is collected and weighed and, therefore, the heat lost in this manner can easily be calculated. Heat lost by radiation and conduction and in the vaporization of water together indicate the total heat elimination. If the heat gained or lost by the body for any given period be added to or subtracted from the heat eliminated the actual amount of heat production can be determined. Unfortunately, the heat change of the body is dependent on the measurement of the rectal temperature which, although the best method at our disposal, does not accurately indicate the heat of the entire body. Direct calorimetry, therefore, in short periods, especially in fever, does not accurately represent the true heat production. Over sufficiently long periods in afebrile conditions it is a satisfactory method and agrees with the heat as determined by the indirect method. It is on this method of indirect calorimetry that one should place the chief reliance in short periods. This has been fully described in the first paper of this series.[10]

In many of the older instruments the production of carbon dioxid alone was measured. In the more accurate of these this determination gives a fairly good index of the height of metabolism.

A consideration of the respiratory quotients is important in studying the mechanism of the rise and fall of temperature, as they indicate the kinds of foodstuffs burned in the body. It has been noted that during shivering glycogen is burned rapidly.[11] If this be true the quotients should be high during a chill.

Another problem that can be considered during these studies is the relation of the heat lost in the vaporization of water to the total heat production. In previous work on this problem the heat of vaporization has always been considered in relation to the total heat produced rather than to the heat eliminated. In afebrile conditions the heat production and the heat elimination are equal. It does not matter, therefore, to which the heat of vaporization is compared. In fluctuations of fever, however, the heat production and heat elimination are unequal. It will be interesting to determine whether the amount of heat lost in vaporizing water follows the level of heat production or that of heat elimination.

HISTORIES OF PATIENTS

Case 1.—Victor J., tertian malaria.

History.—A fireman, born in Sweden, 24 years of age. He was admitted April 6, 1913; discharged cured April 19, 1913. He lived in the South during

10. Lusk, Graham: Clinical Calorimetry, Paper 1, A Respiration Calorimeter for the Study of Disease, The Archives Int. Med., 1915, **15,** 793.

11. Lusk, G.: The Influence of Cold Baths on the Glycogen Content of Man, Am. Jour. Physiol., 1911, **27,** 427.

the year preceding (October, 1912) and had malaria continually. Since that time he has been in New York and has had no trouble until the present attack. He drinks an occasional glass of beer. He denies venereal infection.

For one week preceding admission he did not feel well, but continued work until April 4, when he had a severe chill, followed by high fever and sweating. He was able to work the following day but on the 6th had another chill which brought him to the hospital.

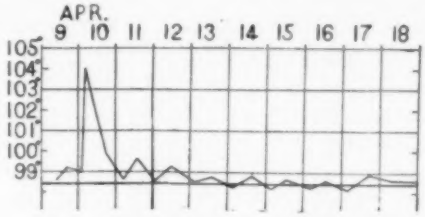

Fig. 2.—Temperature chart of Victor J.

Physical Examination.—A medium sized, well nourished young man. Spleen is palpable two finger breadths below costal margin. On lips and nasal margin there is well marked herpes.

The urine is negative. Blood pressure: systolic, 120 mm.; diastolic, 50 mm. Blood: hemoglobin, 90 per cent.; leukocytes, 9,400; polynuclears, 64 per cent.; malarial organisms, present (tertian type).

He was observed in the calorimeter on April 10 from 8:30 to 11:30 a. m. Quinin was started on the 11th and was continued until the day of discharge, April 19.

Case 2.—George S.; tertian malaria.

History.—A seaman, born in Sweden, 19 years of age. He was admitted March 12, 1917; discharged cured March 22, 1917. In October, 1915, he had gonorrhea which cleared up in six weeks without complications. In June, 1916, he again had a urethral discharge which lasted until October. In September,

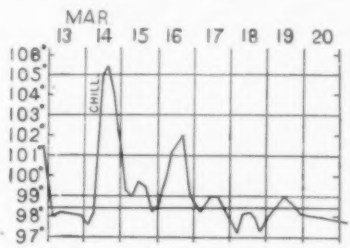

Fig 3.—Temperature chart of George S.

1916, his occupation took him to South America where he contracted malaria. He was treated on his return to New York in St. Vincent's Hospital, where he had several chills. Malarial organisms were found and, after some quinin treatment, he was sent to Bellevue Hospital. While in Bellevue (October) he ran a high, extremely irregular, intermittent fever with no chills, had a moderately enlarged spleen and was very anemic. Erythrocytes, 1,500,000; hemoglobin, 35 per cent.; leukocytes, 4,000; lymphocytes, 70 per cent. After quinin treatment the temperature became normal and the anemia gradually disappeared.

TABLE 1.—

Subject, Date, Weight, Surface Area, Linear Formula	Period	End of Period	Carbon Dioxid, Gm.	Oxygen, Gm.	R. Q.	Water, Gm.	Urine N per Hour, Gm.	Indirect Calorimetry, Cal.	Heat Eliminated, Cal.
Victor J.	Prelim.	8:28	.....	.....	.....	.....	.....	.....	.....
4/10/13									
69.6 Kg.	1	9:28	41.2	39.6	0.76	32.5	0.75	139.2	143.7
1.98 Sq. M.*	2	10:28	39.2	36.5	0.78	33.1	0.75	120.5	145.2
	3	11:28	38.1	35.9	0.77	32.8	0.75	118.3	122.5
Victor J.	Prelim.	9:45	.....	.....	.....	.....	.....	.....	....
4/16/13									
64.0 Kg.*	1	10:45	23.7	24.0	0.72	27.0	0.47	78.2	71.9
	2	11:45	23.4	23.0	0.74	29.4	0.47	75.3	78.7
	3	12:45	25.9	.....	.....	29.2	0.47	.....	80.6
	4	1:45	27.1	27.1	0.72	32.1	0.47	88.4	88.1
George S.	Prelim.	11:18	.....	.....	.....	.....	.....	.....	.....
3/14/17									
71.7 Kg.	1	11:48	13.8	12.7	0.79	19.0	0.55	42.0	36.3
1.83 Sq. M.	2	12:18	13.9	13.3	0.76	18.1	0.55	43.9	37.7
	3	12:40	12.3	10.1	0.88	12.1	0.55	34.2	25.5
	4	1:20	53.5	45.0	0.86	28.8	0.55	153.1	53.7
	5	2:20	43.6	39.5	0.80	48.7	0.55	131.9	91.1
	6	3:20	39.3	37.5	0.76	50.2	0.55	123.9	99.9
Paul K.	Prelim.	12:35	.....	.....	.....	.....	.....	.....	.....
3/16/17									
57.4 Kg.	1	1:35	25.1	23.1	0.79	38.6	0.58	76.6	78.9
1.63 Sq. M.	2	2:35	26.1	24.8	0.77	38.2	0.58	81.9	80.8
Paul K.	Prelim.	10:07	.....	.....	.....	.....	.....	.....	.....
3/17/17									
58.1 Kg.	1	11:07	26.1	24.0	0.79	38.2	0.56	79.3	87.3
1.63 Sq. M.	2	12:17	29.0	28.0	0.75	60.9	0.56	92.0	113.5
	3	1:07	21.8	19.5	0.82	47.4	0.56	65.0	78.2
	4	2:07	26.2	24.3	0.79	47.3	0.56	80.2	89.8
	Aver.	.....	.....	.....	.....	.....	.....	79.1	
Paul K.	Prelim.	7:50	.....	.....	.....	.....	.....	.....	.....
3/20/17									
56.5 Kg.	1	8:50	25.3	23.9	0.77	31.9	0.73	78.2	78.6
1.63 Sq. M.	2	9:50	24.6	22.8	0.79	31.1	0.73	75.1	79.5
	3	10:50	25.8	24.0	0.78	33.7	0.73	78.9	82.0
Fred E.	Prelim.	2:02	.....	.....	.....	.....	.....	.....	.....
3/23/17									
56.7 Kg.	1	3:02	22.4	20.0	0.81	39.0	0.40	66.6	69.4
1.65 Sq. M.	2	4:02	24.0	22.0	0.79	39.1	0.40	72.9	73.2
Sam F.	Prelim.	12:27	.....	.....	.....	.....	.....	.....	.....
4/11/17									
59.5 Kg.	1	1:29	25.4	23.6	0.78	40.0	0.30	78.3	80.2
	2	2:34	59.0	63.5	0.68	63.1	0.30	.....	111.3
Sam F.	Prelim.	3:43	.....	.....	.....	.....	.....	.....	.....
4/12/17									
60.6 Kg.	1	4:43	30.9	28.3	0.79	68.3	0.42	94.3	111.3
1.70 Sq. M.									

—Calorimeter Data

Direct Calorimetry (Rectal Temp.), Cal.	Rectal Temp., C.	Average Pulse	Work-Adder, Cm.	Non-protein R. Q.	Per Cent. Calories from			Calories per Hour		Remarks
					Protein	Fat	Carbohyd.	Per Kg.	Per Sq.M. (Linear)	
.....	40.5	..	..	....	..	..	..	....	.....	Falling temperature
124.0	40.1	..	..	0.75	15	73	12	2.01	65.7*	Used Meeh's formula
120.5	39.6	..	..	0.78	17	63	20	1.86	60.9*	
101.1	39.2	..	..	0.77	17	66	17	1.83	59.7*	
.....	37.1	..	..	....	..	..	..	....	.....	Afebrile
71.5	37.1	..	..	0.70	16	84	..	1.22	39.7*	
75.5	37.0	..	..	0.73	17	77	6	1.18	38.2*	
84.9	37.1	..	..	....						
85.1	37.0	..	..	0.72	14	83	3	1.38	44.8*	
.....	37.3	..	..	....	..	..	..	....	.....	Observation on chill
38.9	37.3	76	2	0.79	17	60	23	1.18	44.9	Motionless
46.7	37.5	..	7	0.75	17	71	12	1.22	48.0	Quiet, turned once
50.8	37.9	96	5	0.88	15	27	58	1.30	51.0	Quiet, mild, shivering last two minutes
168.7	39.9	..	4	0.87	6	42	52	3.20	126.0	Quiet except for chill; chill 12:41 to 1:15
161.3	41.2	100	19	0.80	11	62	27	1.84	72.1	Quiet except last min.
93.4	41.1	..	8	0.76	12	69	19	1.73	67.7	Very quiet
.....	37.7	..	..	....	..	..	..	....	.....	Afebrile
76.6	37.7	77	6	0.78	20	60	20	1.34	47.0	Very quiet
79.9	37.7	75	15	0.75	19	68	13	1.46	50.2	Fairly quiet
.....	38.5	..	..	....	..	..	..	....	.....	Falling temperature
82.9	38.5	80	13	0.80	19	57	24	1.36	48.7	Fairly quiet
103.1	38.3	77	16	0.74	19	73	18	1.35	56.4	Fairly quiet; coughed
65.1	38.0	75	13	0.83	19	48	33	1.34	39.9	Fairly quiet
79.8	37.9	78	16?	0.79	19	58	23	1.38	49.2	Quiet
.....	38.4	..	..	....	..	..	..	....	.....	Fever after chill
72.2	38.2	81	13	0.76	24.9	61	13.6	1.38	47.9	Very quiet
78.2	38.2	77	8	0.78	25.9	55	18.5	1.33	46.1	Very quiet
77.9	38.1	74	15	0.78	24.6	57	17.9	1.40	48.4	Fairly quiet
.....	36.9	..	..	....	..	..	..	....	.....	Afebrile
57.7	36.7	56	10	0.82	16	52	32	1.18	40.3	Fairly quiet
75.7	36.7	56	11	0.79	15	60	25	1.29	44.2	Fairly quiet
.....	37.3	52	..	....	..	..	..	....	.....	Before chill
84.2	37.4	..	19	0.78	11	66	23	1.27	44.6	Slept 15 min.; slightly restless
223.9	39.7	..	27	....	..	..	..	....	.....	Quiet except for chill; chill 1:48 to 2:25
.....	39.1	..	..	....	..	..	..	....	.....	Falling temperature
88.3	38.7	84	16	0.79	12	62	26	1.56	55.5	Quiet; during last 15 min. humidity in calorimeter rose rapidly

Subject, Date, Weight, Surface Area, Linear Formula	Period	End of Period	Carbon Dioxid, Gm.	Oxygen, Gm.	R. Q.	Water, Gm.	Urine N per Hour, Gm.	Indirect Calorimetry, Cal.	Heat Eliminated, Cal.
Sam F. ..... 4/13/17 60.5 Kg. 1.70 Sq. M.	Prelim.	11:30	.....	.....	.....	.....	.....	.....	.....
	1	12:30	23.8	22.1	0.79	38.7	0.38	73.1	75.5
	2	1:30	24.7	23.2	0.77	37.8	0.38	76.7	77.3
	3	1:58	8.8	8.1	0.79	13.3	0.38	26.8	28.6
	4	2:18	11.1	10.9	0.74	13.8	0.38	35.8	31.1
	5	3:18	27.8	25.1	0.81	33.4	0.38	83.9	74.7
Sam F. ..... 4/30/17 61.9 Kg.	Prelim.	11:23	.....	.....	.....	.....	.....	.....	.....
	1	12:23	22.4	19.5	0.84	29.3	0.43	.....	65.4
	2	1:23	24.0	20.8	0.84	29.3	0.43	.....	69.9
	Aver.	.....	.....	.....	.....	.....	.....	67.5	.....

* Height not measured. Used Meeh's formula.

† Small amount of oxygen admitted from preliminary period. At end of second period, sulphuric acid bubbled over into the sodium bicarbonate can, thus generating CO_2 and heat. The indirect calorimetry is therefore worthless, also heat eliminated is unreliable. This period is included to show the great amount of heat stored in the body during chill.

He remained well until March 2, ten days before admission, when he again had chills, fever and sweats. He had a chill every second day until admission to the hospital. The last chill occurred on the morning of admission day.

Physical Examination.—A well developed, fairly well nourished boy, rather pallid. Tongue shows faint white coat. Heart: Left border of dulness is in the fifth space 14 cm. from the median line; right border, 3 cm. from median line in fourth space. There is a soft blowing systolic murmur at the apex, not transmitted. Pulmonary second sound is accentuated. Spleen: Edge is indefinitely felt two finger breadths below the costal margin.

The urine is negative. Blood: Erythrocytes, 3,250,000; hemoglobin, 60 per cent. (Sahli); leukocytes, 4,200; polynuclears, 37 per cent.; lymphocytes, 32 per cent. Many malaria parasites (tertian) are found.

On March 14 he was in the calorimeter. He had a severe chill followed by an abrupt rise in temperature to 105.4 F. On the 15th he was comfortable. On the 16th he had a rise in temperature to 102 F., but no chill. Quinin medication was started on the evening of March 16 and was continued until March 19. He had no further symptoms and was discharged on March 22.

CASE 3.—Paul K.; malaria (aestivo-autumnal).

History.—A laborer, born in Austria, 30 years of age. He was admitted, March 11, 1917, to the service of Dr. R. J. Carlisle, Bellevue Hospital, and was transferred to the Metabolism Ward March 15, 1917; discharged improved March 28, 1917. He drinks moderately of beer and whisky. He denies venereal infection. He came to this country in 1913 and worked in New York for one year. He then moved to North Carolina. Six months later he was seized with chills and fever which came every second day. For eight days he was in a hospital. On his discharge he returned to New York and has had no recurrence until the present time.

On March 6 at 3 o'clock in the morning he was seized with fever and a slight chill. During the following five days he had repeated chills and a constant, severe abdominal pain localized in the left hypochondrium over the spleen.

Direct Calorimetry (Rectal Temp.), Cal.	Rectal Temp., C.	Average Pulse	Work-Adder, Cm.	Non-protein R. Q.	Per Cent. Calories from			Calories per Hour		Remarks
					tein	Fat	hyd.	Kg.	(Linear)	
.....	36.9	..	..	....	..	..	..	....	.....	Time of expected chill
73.1	36.8	55	7	0.78	14	64	22	1.21	43.0	Very quiet; slept 15 min.
77.8	36.8	..	15	0.77	13	69	18	1.27	45.1	Quiet
32.5	36.9	..	4	0.78	15	62	23	1.16	41.1	Quiet
35.5	37.0	..	5	0.73	12	82	6	1.36	50.7	Quiet; felt chilly 2:00 to 2:30 p. m.
87.4	37.3	..	12	0.81	12	58	30	1.39	49.4	Quiet; drank water
.....	36.9	..	..	....	..	..	..	....	.....	Basal (after cure)
38.4	36.8	55	..	....	..	..	..	....	.....	Very quiet; slept 15 min.
69.0	36.7	..	2	....	..	..	..	....	.....	Very quiet
.....	....	..	..	0.85	17	43	40	1.09	39.7	

Physical Examination.—March 16; a moderately emaciated but well developed man, very pallid. Mucous membranes very pale. Herpes on both eyes and nose. Tongue is moist and covered with a moderate white coat. He coughs frequently. A few râles are heard over both sides of the chest. The spleen is felt as a soft edge at the costal margin. Cervical, inguinal, epitrochlear and axillary lymph nodes are palpable.

His urine is negative. Blood pressure: systolic, 110 mm.; diastolic, 68 mm. Sputum negative for tubercle bacilli.

He had sharp rises in temperature on March 12 and 14. Neither during these exacerbations nor in those which followed did he have a chill. He was in the calorimeter from 11:30 a. m. to 2:35 p. m., March 16. At that time his

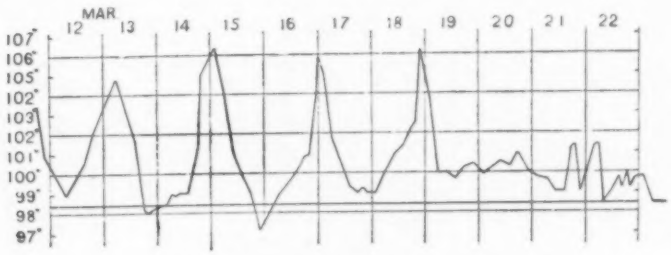

Fig. 4.—Temperature chart of Paul K.

blood showed leukocytes, 4,000; erythrocytes, 2,700,000; hemoglobin, 55 per cent. (Sahli); malarial parasites were found. March 17, from 9:30 a. m. to 2:15 p. m., he was again in the calorimeter. On the 18th quinin sulphate, 20 grains, was given. On the following day parasites were again found in the blood. On the 20th he was observed in the calorimeter from 7 to 10 p. m. Quinin was started again on the 22d and was continued until the time of discharge, March 28. On the 22d leukocytes were 7,000; erythrocytes were 2,200,000; hemoglobin (Dare), 30 per cent.; anisocytes and poikilocytes were present. At the time of his discharge he had a normal temperature and had no complaints.

TABLE 2.—Calorimeter Data in Malaria

Subject and Date	Age	Period of Disease	Rectal Temp., C.	Average Pulse	Average R. Q.	Indirect Calorimetry Average per Hour		Per Cent. Divergence of Direct Cal. from Indirect Cal.	Per Cent. Total Heat Elimination Lost in Vap. of Water	Change in Rectal Temperature	Change in Average Body Temperature	Per Cent. Rise Above Average Normal Basal for Age
						Calories per Kg.	Calories per Sq. M.					
Paul K. 3/16/17	30	Afebrile; between paroxysms	37.7-37.7	77	0.78	1.4	48.5	−1.0	29	±0.0	±0.0	+22
George S. 3/14/17	19	Afebrile; one hour before severe chill	37.3-37.5	76	0.78	1.2	46.5	−0.2	29	+0.2	+0.2	+14
Sam F. 4/11/17	32	Afebrile; one hour before severe chill	37.3-37.4	52	0.78	1.3	44.6	−7.0	29	+0.1	±0.0	+12
Sam F. 4/13/17	..	.....	36.9-36.9	55	0.78	1.2	43.7	+3.4	29	±0.0	−0.1	+ 9
Sam F. 4/30/17	32	Afebrile; after treatment 17 days after last chill	36.9-36.7	55	0.84	1.1	39.7	−6.3	26	−0.2	±0.0	± 0
Fred E. 3/22/17	18	Afebrile; after treatment 6 days after last chill	36.9-36.7	56	0.80	1.2	42.3	−5.0	32	−0.2	−0.1	± 0
Victor J. 4/16/13	24	Afebrile; after treatment 6 days after last chill	37.1-37.0	..	0.72	1.3	41.2*	−4.8	..	−0.1	−0.1	+14
Paul K. 3/20/17	30	Late falling temperature after end of last paroxysm	38.4-38.1	78	0.78	1.4	47.4	−1.8	24	−0.3	−0.2	+19
George S. 3/14/17	19	Rising temperature before chill	37.5-37.9	96	0.88	1.3	51.0	+13.5	28	+0.4	+0.2	+24
George S. 3/14/17	19	Chill	37.9-39.9	..	0.86	3.2	126.0	+9.3	31	+2.0	+1.7	+216
George S. 3/14/17	19	Rising temperature after chill	39.9-41.2	100	0.80	1.8	72.1	+22.0	31	+1.3	+1.7	+76
George S. 3/14/17	19	High constant temperature after chill	41.2-41.1	..	0.76	1.7	67.7	−24.6	29	−0.1	+0.4	+65
Victor J. 4/10/13	24	Falling temperature immediately after chill	40.5-39.2	..	0.77	1.9	62.1	−7.0	..	−1.3	−0.8	+81
Sam F. 4/12/17	32	Falling temperature, profuse sweating	39.1-38.7	84	0.79	1.6	55.5	−7.0	36	−0.4	−0.3	+40
Paul K. 3/17/17	30	Late falling temperature	38.5-37.9	80	0.79	1.4	48.5	+0.5	34	−0.6	−1.1	−23

* Meeh's formula.

TABLE 2-A.—Calorimetry Data After Food

Name and Date	Body Weight, Kg.	Food				Urine N	Feces N, Estimated	Excreta N	Nitrogen Balance	Urine Volume, C.c.	Length of Period, 24 Hours
		Total Calories	Carbohydrate, Gm.	Fat, Gm.	Nitrogen, Gm.						
George S.											
March 14	....	1,089	85.5	63.9	5.6	11.2	1.1	12.3	−6.2	865	19 hours 2 minutes
March 15	....	2,690	278.0	130.0	13.4	17.8	1.3	19.1	−5.7	1,070	23 hours 45 minutes
March 16	....	2,900	323.0	128.0	14.9	14.1	1.5	15.6	−0.7	1,070	24 hours 15 minutes
March 17	70.4	3,000	340.0	131.0	15.0	15.3	1.5	16.8	−1.8	1,340	23 hours 45 minutes
March 18	69.8	2,950	308.0	140.0	15.1	13.5	1.5	15.0	−0.1	1,190	
March 19	....	3,300	384.0	144.0	15.1	14.4	1.5	15.9	−0.8	1,080	
Paul K.											
March 16	57.4	1,750	171.0	86.0	9.6	19.4	1.0	20.4	−10.8	1,130	
Fred E.											
March 18	56.9	2,470	248.0	115.0	15.0	13.2	1.5	14.7	+0.3	890	
March 19	56.5	2,500	258.0	114.0	15.1	12.1	1.5	13.6	+1.5	1,030	
Sam F.											
April 11	59.5	1,230	119.0	57.0	8.2	12.2	...	13.0	−4.8	580	
April 12	60.6	1,790	178.0	83.0	11.3	14.8	1.18	15.9	−4.6	865	
April 13	60.5	1,885	180.0	97.0	9.6	13.0	1.0	14.0	−4.4	1,180	
April 14	....	2,470	240.0	119.0	14.9	14.6	1.5	16.1	−1.2	1,640	
April 15	60.9	2,415	242.0	112.0	14.7	16.2	1.5	17.7	−3.0	1,510	
April 16	60.7	2,510	241.0	123.0	15.1	12.5	1.5	14.0	+1.1	1,020	
April 17	....	2,530	249.0	122.0	14.7	12.5	1.5	14.0	+0.7	1,340	
April 18	60.6	2,450	240.0	117.0	14.8	11.4	1.5	12.9	+1.9	1,100	

Case 4.—Fred E.; tertian malaria.

History.—A farmer's helper, born in the United States, 18 years of age. He was admitted March 16, 1917, and discharged improved March 28, 1917. While in Mexico in 1912 he contracted malaria and has had recurrences every spring lasting from two to four weeks. During the first three attacks the chills occurred every second day. For the past two years, however, he has had a chill every morning during the attacks.

March 13, three days prior to admission, he was seized with a chill followed by high fever which lasted two to three hours. A similar paroxysm occurred on the two following mornings. He had a very severe headache and a frequent, painful cough. During the morning of the admission day he had a severe chill.

Physical Examination.—A short, moderately well nourished boy. He is pallid and his face is covered with pimples. The tongue shows a slight white coat. The tonsils are moderately enlarged. There is no general lymphatic enlargement. Heart: The left border of dulness, in the fifth space, is 13.5 cm. from the median line; the right border is in the fourth space 4 cm. from the median line. A harsh systolic murmur is heard in the third and fourth spaces half way between the median line and the left border. The spleen is very much enlarged downward and toward the median line, 10 cm. below the costal margin in the left midclavicular line.

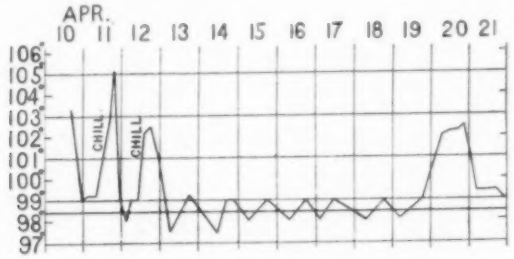

Fig. 5.—Temperature chart of Sam F.

His urine was negative. March 17, erythrocytes were 3,912,000; leukocytes 4,200; polymorphonuclears, 71 per cent.; lymphocytes, 17 per cent.; large mononuclears, 7 per cent., and malarial parasites of tertian form were found.

At the time of admission, the afternoon of March 16, the temperature was still elevated (101.8 F.) from the chill of the morning. It reached normal the following morning and remained so until the time of discharge. March 23 he was observed in the calorimeter for three hours. He was given quinin for the first time March 25, and March 28 was sent to a convalescent home.

Case 5.—Sam F.; tertian malaria.

History.—A seaman, born in Sweden, 32 years of age. He was admitted April 9, 1917, to the service of Dr. Alexander Lambert at Bellevue Hospital; transferred to the metabolism ward April 10 and discharged cured April 30, 1917. He had gonorrhea with epididymitis nine years prior to admission. At the same time he had soft chancres with resulting bubo which, however, did not suppurate. He denies syphilis. He drinks three or four glasses of beer a day; no whisky.

In August or September, 1916, while in Java, he developed malaria. He had chills every second day during the trip from Java to Genoa, Italy. On his arrival in Genoa he went to a hospital where he was treated until March 4, 1917. During most of the time he had no chills. He continued treatment until

March 20. He arrived in this country March 27 and had the first recurrence of chills April 9, the day of admission to Bellevue Hospital.

Physical Examination.—A fairly well nourished, muscular, young man. He has a moderate dorsal kyphosis with right lateral scoliosis. The tongue shows a slight white coat. The tonsils are large. The spleen is felt three finger breadths below the costal margin. The edge is hard, firm and rather thick. On the skin of the prepuce there are two small, nonindurated ulcers, probably due to filth.

Urine: Negative, except for a few pus cells. Blood: April 9, 1917, erythrocytes, 4,600,000; hemoglobin, 90 per cent. (Sahli); leukocytes, 4,600; Wassermann, negative. Gonococcus fixation test, negative.

He had a severe chill April 10 and another April 11, during which he was observed in the calorimeter. April 12, at 1:50 p. m., he had a chill lasting forty minutes. He was in the calorimeter from 3 to 5 p. m. April 13 he did not have a chill although no quinin had yet been given. He was observed in the calorimeter during the time of the expected chill, 11 a. m. to 3 p. m. On the evening of the same day he received quinin, which was continued until April 26, when he complained of ringing in the ears. He was rapidly convalescing when, on the 19th, he developed a rather severe tonsillitis. From this he recovered rapidly and was discharged as cured on April 30.

V. DISCUSSION OF EXPERIMENTS

(*a*). *Basal Metabolism in Afrebrile Periods.*—In all, seven observations were made on the patients when they were without fever. Four of these were in the intervals between the paroxysms; three after treatment.

TABLE 3.—OBSERVATION DURING AFEBRILE PERIODS

Name and Age	Period of Disease	Calories per Sq. M. per Hour	Surface Area Formula	Per Cent. Deviation from Average Normal
Paul K.	Between paroxysms	48.5	Lin.	+22
George S.	1 hour before severe chill	46.8	Lin.	+14
Sam F.	1 hour before severe chill	44.6	Lin.	+12
Sam F.	2½ hours before expected chill	44.0	Lin.	+ 9
Average				+14
Sam F.	After treatment	39.7	Lin.	± 0
Fred E.	After treatment	42.3	Lin.	± 0
Victor J.	After treatment	38.9	Meeh	+14
Paul K.	After treatment	47.4	Lin.	+10

Paul K. was never seen to shiver while in the hospital. As is shown by the temperature chart, however, he had unusual periodic rises in temperature. The observation was made twenty-eight hours after the temperature induced by the last paroxysm had reached normal. The very marked increase in metabolism in this case may have been due in part to the very profound secondary anemia. His erythrocytes were 2,200,000, with a hemoglobin of 30 per cent.

Two observations were made on Sam F. when afebrile and before treatment. The first, April 11, was made during the hour preceding a severe chill. The second observation, April 13, was made during the two and a half hours

preceding an expected chill. The observation of that day lasted five hours and was continued through the time of the expected chill. During the last two hours, however, there were chilly sensations, with a slight rise in temperature. Only the first two and a half hours, therefore, were considered as basal. Seventeen days after the last paroxysm, four days after the end of vigorous quinin treatment, he was again observed and was found to have a normal heat production.

Fred E., who had suffered with annual attacks of malaria for five years, who had a large, firm spleen and a moderate secondary anemia, was examined seven days after the last chill. It was suspected that, since there was an increased metabolism between chills, there might be a similar increase in the condition of malarial cachexia. No change in metabolism was found, the figure, 42.3 calories per square meter per hour, being normal for a boy of 19.

The observation on Victor J. was made seven days after the last chill. Only the first two hours of the four-hour observation are included in the basal average. During the last two hours the patient was very restless. There is some doubt as to whether the first portion can be considered as basal because of possible restlessness of the patient. Since this experiment was made before the linear formula was devised, the results are expressed in terms of Meeh's formula and compared with 34.2 calories per square meter of surface per hour, the average normal for this formula.

From a consideration of the above experiments it is seen that the total metabolism was raised 14 per cent. in the afebrile period between chills. If the results on Paul K. were to be excluded on account of the complicating factor of anemia, the figures would still be 12 per cent. above the average basal level. Since there was no fever, this increase must be attributed to the nature of the disease itself.

Two of the observations on patients after treatment showed a normal metabolism. The third case, Victor J., showed an increase of 14 per cent. As there are no detailed notes on this observation, and as he was somewhat restless, this can scarcely be included in the basal average.

(*b*) *Metabolism During Paroxysms.* — In considering the calorimetric aspects of a malarial paroxysm it is well to subdivide it into six phases:

1. A basal period for an hour or so before the chill.

2. A short period of rising temperature immediately before the chill which might be called the *prodromal phase*.

3. The period of the chill itself.

4. A period of rising temperature following the chill.

5. The period of high, continuous temperature, which corresponds to the clinical stage of heat.

6. The period of falling temperature, which might be still further subdivided into the early and late fall of temperature.

Six observations were made on the different phases of temperature during the malarial paroxysm: one on the rising temperature before, during and after the chill, including one hour of high, continuous temperature; one on the chill and the period immediately preceding; two on the early fall in temperature, and one on a later fall when the temperature was nearly normal. A single experiment was made on the

continuous temperature which persisted after the end of the last paroxysm on Paul K.

In considering these experiments four main questions of the mechanism of the rise and fall of body temperature will be investigated and discussed:

1. The relation of heat production to heat elimination in the rise and fall of temperature.

2. The divergence of the direct heat production as indicated by the measurement of rectal temperature from that determined by the method of indirect calorimetry.

3. The varying relation during the different phases of temperature of the heat lost in the vaporization of water to the heat production, on the one hand, and the heat elimination on the other.

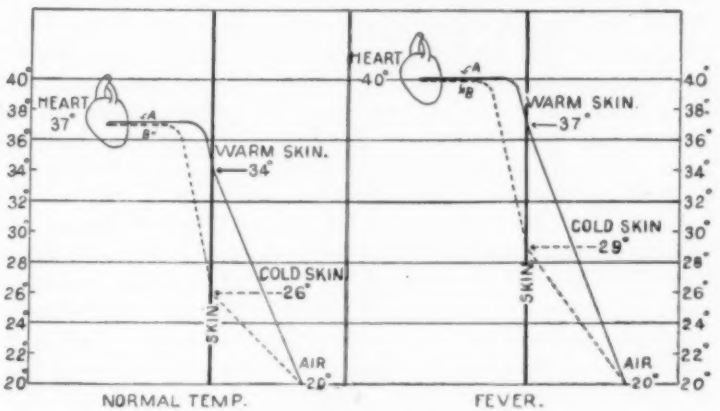

Fig. 6.—Scheme of body heat loss at different levels of temperature.

4. The influence of body temperature on the heat production.

The respiratory quotients will be mentioned as indicative of the foodstuffs burned in the different phases of fever.

(*c*) *Heat Elimination in Fever.*—In considering the question of heat elimination two questions naturally present themselves: First, how is it possible that two individuals of the same size can eliminate the same amount of heat if one has a cool skin and the other a warm skin? Second, how can one explain the fact that the heat elimination of a malarial patient remains almost exactly the same per hour during the period before the chill, during the period of the chill, and during the period of high, continuous temperature immediately after the chill?

The phenomena can be explained with the aid of the diagrams (Fig. 6). It is obvious that if the temperature of the interior of the

body, as represented by the heart or the deep rectal region, be 37 C., and the outside air be 20 C., there must be a sharp drop somewhere between these two points. This can be represented schematically in curves which give the temperature gradients under varying conditions. Of course, the temperatures and distances from the skin selected for the charts are not exact, since no actual measurements have been made in these cases.

Let us consider the diagram at the left, Figure 6, which shows the gradients that would be found in an individual with a rectal temperature of 37 C. exposed to an atmosphere at 20 C. Curve A represents the conditions when his skin is warm with, say, a skin temperature of 34 C. The interior of the body, up to a point within 1 or 2 cm. of the skin, has a fairly uniform temperature and the blood is cooled, either in the skin itself, or perhaps, a centimeter beneath the skin; in other words, the sharp knuckle of the curve representing the sudden fall in temperature lies just beneath the skin. The dotted line B represents the same individual with the same rectal temperature exposed to an environmental air of 20 C. In this second case, however, the skin is cold; say the surface temperature is 26 C. The greatest cooling of the blood takes place at a considerable depth beneath the skin, perhaps 2 or 3 cm. In both cases the curves start and end at the same point. In both cases the individual produces within his body the same amount of heat per hour and eliminates the same amount of heat per unit of surface. In the first instance the cooling is near the surface, and in the second it is deep beneath the skin. In the first instance the subcutaneous arterioles are dilated and the warm blood comes in contact with the cooler air. In the second instance the surface arterioles are contracted, the subcutaneous tissues are cold, and these cold subcutaneous tissues cool off the blood circulating freely in the deeper structures.

In fever or with a changing temperature the phenomena are similar. If the internal temperature, for instance, be 40 C., the skin temperature will probably be correspondingly higher. The curves representing conditions with warm skin and cold skin start and end at the same points, but the knuckles in the curves representing the sudden drops in temperature are similar to the knuckles in Figure 6.

This brings us to a discussion of the average body temperature, a matter of great theoretical interest. Probably the average body tempeature will never be determined exactly except under very unusual conditions. Some very interesting studies on this subject have been made by Benedict and Slack,[12] working on man, and Henriques,[13]

12. Benedict, F. G., and Slack, Edgar P.: A Comparative Study of Temperature Fluctuations in Different Parts of the Human Body, Pub. 155, Carnegie Institution of Washington, 1911.

13. Henriques, V., and Hanson, C.: Skand. Arch. f. Physiol., 1901, **11,** 161.

working on pigs. These observers found that the temperature of the interior of the body was fairly uniform until a point was reached 4 or 5 cm. from the surface. Then there was a gradual cooling until just beneath the surface, where the temperature was several degrees lower than in the interior. Other observers have found, also, that some of the internal organs have temperatures averaging higher than others. While the temperature taken deep in the rectum is, perhaps, the best single spot to use for the estimation of the body temperature, it is obvious that the actual rectal temperature is higher than that of the whole body, since a large portion of the body lies close to the surface. If, for instance, a man weighed 70 kg., there might be as much as 15 kg. of tissue within 1 cm. of the surface, and the temperature of this surface tissue might, on the average, be 2 degrees cooler than the interior of the body. It is obvious no exact calculations can be made. For the purpose of drawing curves, however, we can arbitrarily consider that the average body temperature is half a degree below that of the rectum. If the skin and subcutaneous tissue should grow colder the average body temperature would be even farther than this below the rectal temperature.

While it is impossible to determine the absolute average body temperature, we can calculate the changes in the average body temperature by using a new method. This is made possible by the great accuracy of the modern respiration calorimeter. We know that in a long series of experiments the methods of direct and indirect calorimetry agree very closely. Experiments have shown that the method of indirect calorimetry is accurate within any comparatively short period, and by this method we can find the heat produced within the body during the experimental period. Alcohol checks and electric checks have shown that the measurement of the heat eliminated from the body is determined accurately by the calorimeter. If, therefore, in any given period we can determine the calories produced in the body and the calories eliminated from the body, the difference between these two represents the calories either stored in the body or lost from its mass of tissue. If we know the weight of the body we can calculate its hydrothermal equivalent by multiplying the kilograms by the average specific heat of the body. This has usually been assumed to be 0.83, although, as we have pointed out before, the figures may be somewhat lower, especially in obese individuals.[14] For our purposes the figure 0.83 is close enough to enable us to determine with a fair degree of accuracy the heat equivalent of the body in

14. See Gephart, F. C., and Du Bois, E. F.: Clinical Calorimetry, Paper 4, The Determination of the Basal Metabolism of Normal Men and the Effect of Food, THE ARCHIVES INT. MED., 1915, **15,** 835.

terms of kilograms of distilled water. If we know the calories stored in the body during a given period and the hydrothermal equivalent of the body, we can easily determine the rise or fall in the average body temperature during a given period. If, for instance, during a certain period of the experiment, the heat production, as measured by indirect calorimetry, should be 100 calories and the heat elimination 60 calories, this would mean a storage of 40 calories within the tissues. If the body weight were 70 kg., this figure multiplied by the factor 0.83, which represents the specific heat of the body, would indicate that the body, from a thermal standpoint, was equivalent to 58.1 kg. of distilled water. If 58.1 calories had been stored in the body, the average temperature would have risen 1 degree C., but since only 40 calories were stored in the body, it is obvious that the average temperature rose approximately 0.66 degree C. In a similar fashion, if the heat elimination exceeds the heat production, it is obvious that

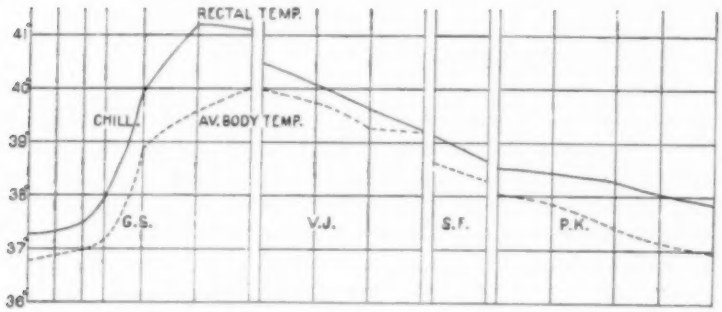

Fig 7.—Comparison of changes in rectal temperature and average body temperature. Curves constructed from experiments on four subjects in different stages of the malarial paroxysm. The continuous line shows the rectal temperature; the dash line indicates the changes in the average body temperature which, for purposes of calculation, is assumed to be one half a degree lower than the rectal temperature at the start of each observation.

the average body temperature must be falling. This calculation has been made in all the experiments. We know the rectal temperature at the start of the experiment and, for the purposes of calculation, the average of body temperature has been assumed to be a half a degree lower. If, for instance, in the above period the rectal temperature at the start had been 37.0 C., we would assume that the average body temperature was 36.50 C. At the end of the period the average body temperature would have been 37.16 C. Perhaps the rectal reading at this same time might have been 38.1. In a similar fashion the change in the average body temperature in the next period would have been calculated, using as a starting point the figure 37.16.

This is a roundabout method of calculating temperature changes, but it is believed to be more accurate than any system which depends on a single thermometer in a place like the rectum, where it can be moved from side to side or can be embedded in feces, or in a place like the axilla, groin or mouth, where the thermometer may be affected by a partial opening of the cavities.

In the following discussion we shall compare the average body temperature as obtained by this method with the rectal temperature. In this way we can obtain some idea of the accuracy of rectal temperature as an index of the temperature of the body during a paroxysm.

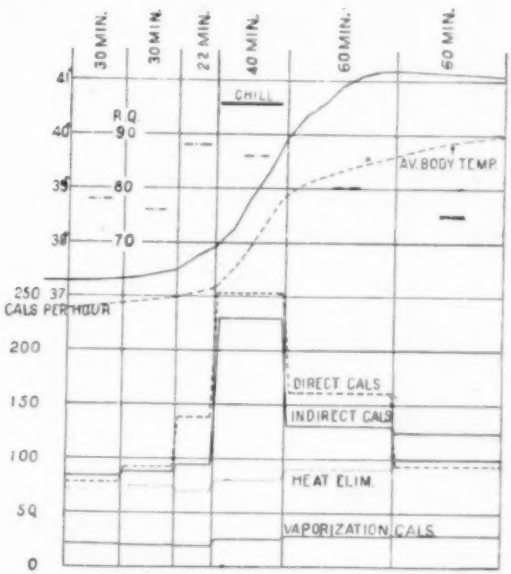

Fig. 8.—Metabolism chart of George S., March 14, 1917.

(*d*) *Individual Experiments During Paroxysms.*—

George S., March 14, 1917, Chart 8. The curves show the relationship of heat production and heat elimination before, during and after a malarial chill. The uppermost line shows the changes in rectal temperature. The accompanying dash line shows the changes in the average body temperature. The middle dash line represents the heat production in calories as measured by the direct method; the solid line, the heat production by the indirect method; the dotted line, the heat eliminated, and the lowest solid line, the calories lost in the vaporization of water. The respiratory quotients are represented above the rectal temperature by dashes for each period.

Phase I.—Normal temperature (Periods 1 and 2). The metabolism is 14 per cent. above the average basal level for a boy of 19. The direct and indirect calories agree exactly. The rectal temperature indicates a rise of 0.2 degree C.,

which corresponds exactly to the increase in average body temperature. The total heat elimination is at a level slightly below the heat production. The heat lost in vaporization is 29 per cent. of the total heat eliminated; 26 per cent. of the heat produced (direct method). The respiratory quotient is normal.

Phase II.—Rising temperature before chill (Period 3). Heat production is 21 per cent. above basal level. The direct method indicates a higher metabolism. The rectal temperature shows a rise of 0.44 degree C. The average body temperature rises only 0.15 degree C. Heat elimination does not change from its former level. The heat lost in vaporization of water is 28 per cent. of the total heat elimination; only 14 per cent. of the direct heat produced. The respiratory quotient rises to 88.

Phase III.—Violent chill (forty minutes) (Period 4). Heat production is 216 per cent. above the average normal level. The heat production as measured by the direct method is higher than is indicated by the indirect. The rectal tem-

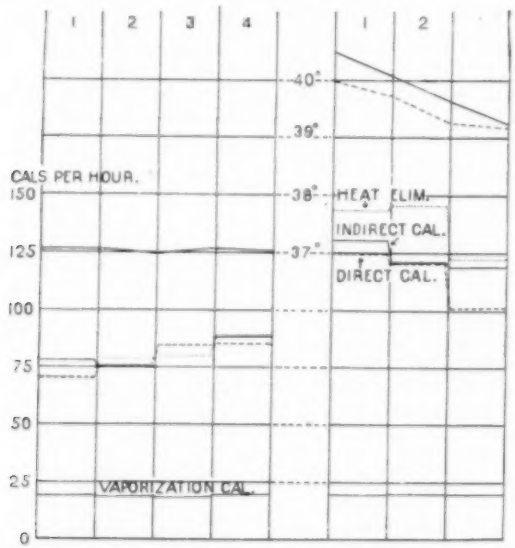

Fig. 9.—Metabolism chart of Victor J., April 10 and April 16, 1913.

perature shows a rise of 2.0 degrees C.; the average body temperature 1.7 degrees C. The total heat elimination is raised very slightly. The heat lost in vaporization of water is 31 per cent. of the total heat eliminated; only 10 per cent. of the heat produced (direct method). The respiratory quotient is high, 0.86.

Phase IV.—Rising temperature following chill (Period 5). Heat production drops to 80 per cent. above the normal basal level. The heat production, as measured by the direct method, is higher than is indicated by the indirect. The rectal temperature shows a rise of 1.3 degrees C.; the average body temperature only 0.7 degree C. The total heat elimination has risen to a higher level. The heat lost in the vaporization of water is 31 per cent. of the total elimination; 18 per cent. of the heat produced (direct method). The respiratory quotient is 0.80.

Phase V.—High, continuous temperature (Period 6). Heat production is 71 per cent. above the normal basal level. The heat production as measured by the direct method is not so high as is indicated by the indirect method. The rectal

temperature shows a fall of 0.1 degree C.; the average body temperature a rise of 0.4 degree C. The total heat eliminated has again risen and is now higher than the heat produced as measured by the direct method. Heat lost in vaporization is 29 per cent. of the total heat eliminated; 31 per cent. of the heat produced (direct method). The respiratory quotient is 0.76.

Victor J., April 10, 1913, Chart 9. The curve in Figure 9 shows the relationship between heat production and heat elimination during early fall in temperature. The lines correspond to those on Chart 8. The left hand portion of the chart shows the results obtained on a day without fever. On right is the observation of three hours in length, immediately after the chill. Heat production is 81 per cent. above the average basal for a male adult. The heat production as measured by the direct is not so high as is indicated by the indirect. The rectal temperature indicates a drop of 1.1 degrees C.; the average body temperature only 0.8 degree C. The total heat eliminated is higher than the heat produced. The percentage of heat lost by vaporization is not accurately measured. At the time of the observation (1913) ventilation was not sufficient to remove the water. Respiratory quotients average 0.76.

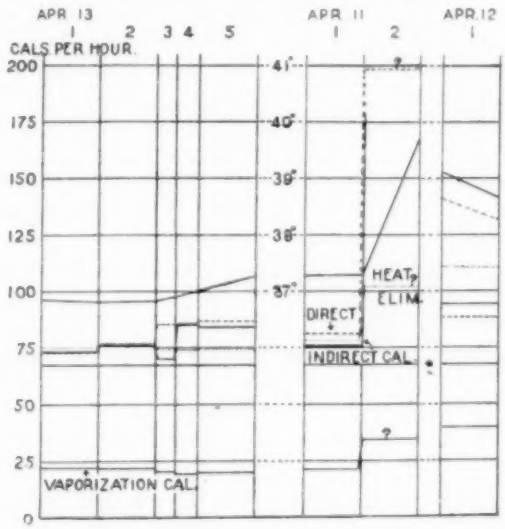

Fig. 10.—Metabolism chart of Sam F., April 10, 11, 12, 1917.

Sam F. The curves in Figure 10 show the relationship of heat production and heat elimination before, during and after chill. The lines correspond to those on Figure 8. The chart includes three observations made on April 11, 12 and 13, 1917.

Phase I.—Normal temperature before expected chill (Periods 1 and 2). Heat production is 11 per cent. above the average basal level. Direct and indirect methods agree. Both rectal and average body temperature remain constant. Total heat elimination corresponds to heat production. The respiratory quotient is 0.78.

Phase II.—Rise in temperature before expected chill (Period 3). Heat production by the indirect method 3 per cent. above the average basal level. Heat production by the direct method rose slightly: Average body temperature

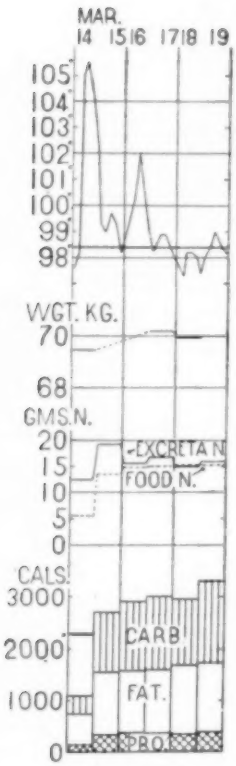

Figure 11

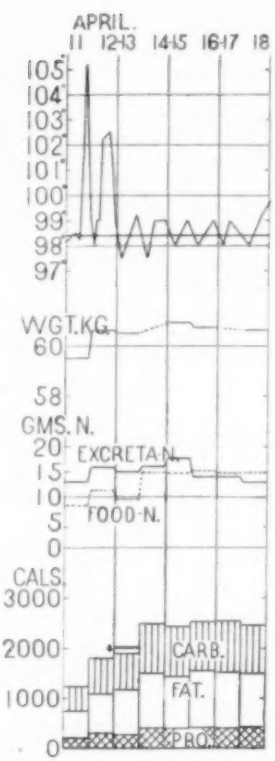

Figure 12

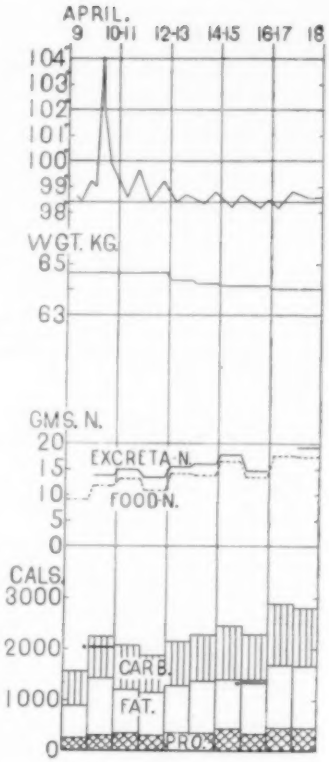

Figure 13

Fig. 11.—George S. Temperature, weight, nitrogen balance and food chart.

* Small dot and heavy horizontal line at level of about 2,200 calories show estimated heat production for twenty-four hours.

Fig. 12.—Sam. F. Temperature, weight, nitrogen balance and food chart.

* Small dot and heavy horizontal line at level of about 2,000 calories show estimated heat production for twenty-four hours.

Fig 13.—Victor J. Temperature, weight, nitrogen balance and food chart.

* Small dots and heavy horizontal lines at level of about 2,000 calories April 10, and at about 1,200 calories April 16, show estimated heat production for twenty-four hours.

dropped slightly; rectal temperature rose 0.1 degree C. Heat elimination did not change its level. The respiratory quotient was unchanged.

Phase III.—Chilly sensations, no chill (Periods 3 and 4). Heat production 27 per cent. above the average basal level. The direct and indirect methods agree. The rectal temperature shows a rise of 0.3 degree C. The average body temperature shows a corresponding rise. Heat elimination does not rise with production but remains at its former level.

Observation made on April 11, 1917: Phase I (Period 1).—Normal temperature preceding severe chill. Heat production is 12 per cent. above the average

basal level. Indirect calorimetry not entirely reliable because of a slight amount of oxygen admitted from the preliminary period. Heat elimination at corresponding level.

Phase III.—Severe chill. The results were ruined by an accident caused by enormous increase in metabolism (sulphuric acid bubbled into the sodium bicarbonate can, generating carbon dioxid, water and heat). The period is included to show the large amount of heat stored in the body during the chill.

Observation made April 12, 1917: Falling temperature with sweating. Heat production is 40 per cent. above the basal level. Heat elimination is tremendously increased. Heat production by the direct method is lower than is indicated by the indirect. Rectal temperature fell 0.46 degree C.; average body temperature fell 0.33 degree C. The heat lost in vaporization was 36 per cent. of the total heat elimination. This did not include all of the water eliminated for the humidity in the calorimeter began to rise during the last ten minutes of the period.

Paul K., March 17, 1917. Late falling temperature. Heat production was 22 per cent. above the normal level. The production by the direct method was higher than was indicated by the indirect. Rectal temperature fell 0.7 degree C. during the observation. Average body temperature fell 1.1 degrees C. Heat elimination was much higher than heat production. Heat lost in vaporization was 27 per cent. of the total heat eliminated; 26 per cent. of the heat produced (direct method) during the first hour. At the end of the first hour the patient spilled the urine, thus spoiling the results of water vaporization. The respiratory quotients were about 0.79.

Another observation was made on Paul K. March 20, after the end of the last paroxysm, when his temperature was still elevated. On this day his heat production was + 19 per cent., slightly lower than it had been four days before in the afebrile period between paroxysms.

(*e*) *Nitrogen Balances.*—In Figures 11, 12 and 13 will be found graphic representations of the food administered, temperature curve, weight curves and the relationship between food nitrogen and excreta nitrogen.

In the case of George S. it will be seen that there was a negative nitrogen balance during the febrile period in spite of an intake of 3,000 calories a day. The patient came to a nitrogen equilibrium when the malarial paroxysms ceased.

In the case of Sam F. the nitrogen balance remained negative until the fourth day of normal temperature, although his food intake had reached about 2,400 calories on the second day of normal temperature.

Victor J. showed a negative nitrogen balance six days after his last paroxysm, but his intake was little over 2,000 calories.

In general, it will be noted that malarial patients gave evidence of a toxic destruction of protein, just as did the typhoid patients studied in Paper 7 of this series.[15] In spite of caloric intake, which covered the heat production and supplied an ample amount of protein and carbohydrate, there was a negative nitrogen balance.

(*f*) *Direct and Indirect Calorimetry.*—In the observations on malarial patients without temperature, the heat production, as mea-

15. Coleman, W., and Du Bois, E. F.: Clinical Calorimetry. Paper 7. Calorimetric Observations on the Metabolism of Typhoid Patients With and Without Food, THE ARCHIVES INT. MED., 1915, **15,** 887.

sured by the direct method, amounted to 917.1 calories; by the indirect, to 927.1 calories, a total divergence of —1.1 per cent. In the observations in fever the total heat production by the direct method was 1,675.7 calories; by the indirect, 1,664.1 calories, a divergence of +0.7 per cent. In the febrile experiments the variations for the individual hours was often very great. In the period for the chill on George S. the direct calorimetry showed a heat production of 49 per cent. above that shown by the indirect. The greatest divergence for any single observation was 7 per cent. Six of the eleven experiments showed a divergence of 5 per cent. or less. The total heat production for all observations was 2,592.8 calories, as measured by the direct, and 2,591.2 as measured by the indirect, a total divergence of + 0.08 per cent.

TABLE 4.—Percentage Divergence of Direct from Indirect Calorimetry in the Individual Experiments

Percentage Divergence	Number of Experiments Falling in Each Group		
	Plus Divergence	Minus Divergence	Total
0 - 5	2	4	6
5 - 7	2	3	5
	Indirect	Direct	Divergence, per Cent.
Afebrile Experiments	927.1	917.1	—1.1
Febrile experiments	1,664.1	1,675.7	+0.7
Total calories measured in all experiments	2,591.2	2,592.8	+0.08

(*g*) *Character of Foodstuffs Burned.*—All of the respiratory quotients were within normal limits, the highest being 0.88; the lowest 0.72. Before and during the chill on George S. the quotients were higher than in any of the other periods. This shows an increased combustion of carbohydrate and would indicate a mobilization of the glycogen stores of the body. It is particularly interesting that the quotient rose before the chill and even before there was any noticeable increase in metabolism. As this period was only twenty-two minutes in length, it is unsafe to draw from it any conclusions.

The close agreement between the direct and indirect methods during the observations, the consistently normal respiratory quotients, indicate that there is no change in the metabolism of malaria sufficiently profound to upset calculations based on the method of indirect calorimetry.

(*h*) *Relation of Heat Production to Heat Elimination.*—From a consideration of the preceding experiments one relation stands forth with distinctness. The comparison of heat elimination and heat production shows unmistakably that the rise in temperature is chiefly dependent on an increase in heat production and that the heat elimination does not change. The temperature is maintained at a high level by a heat production which is still high, and an elimination which, although rising, is not adequate to take care of the heat produced. The fall in temperature is accomplished by a combination of two factors, a decrease in heat production and an increase in heat elimination. Of the two factors, however, the increase in elimination is much more striking.

(*i*) *Periods of Malarial Paroxysms.*—A malarial paroxysm has usually been divided into three stages: first, that of heat; second, that of fever; third, that of sweating. On closer analysis this rough clinical division is not satisfactory for a description of the changes in heat regulation. The following subdivision into six stages gives one a better chance to describe the phenomena observed in the calorimeter experiments:

1. *Preliminary Period.* The rectal temperature remains constant and there is no warning of the coming paroxysm.

2. *Prodromal Period.* Fifteen or twenty minutes before the chill the rectal temperature begins to rise a little. The patient begins to feel a little uncomfortable.

3. *Period of Chill.* This may last a few minutes or as long as an hour. The rectal temperature rises abruptly. The surface of the body may become relatively and perhaps actually cooler. The average body temperature, therefore, rises somewhat less abruptly than the rectal temperature. The patient feels cold and shivers violently.

4. *Period of Rising Temperature After the Chill.* The rectal temperature rises less rapidly than during the chill. The surface of the body becomes warmer. The patient feels weak and exhausted after his chill.

5. *The Period of High, Continuous Temperature.* The rectal temperature changes but little. The surface temperature rises steadily. The patient feels hot.

6. *Period of Falling Temperature.* The rectal temperature falls much more gradually than it rose. During the very earliest stage of this period the surface temperature is still rising. Soon it begins to fall, but not so rapidly as the internal temperature. In the latter part of the period the two fall at about the same rate. The patient sweats profusely.

Heat Production: In the preliminary period this is unchanged. In the short prodromal period there may be an insignificant rise. During the chill the violent muscular exercise increases heat production 100 to 200 per cent.[16] Immediately after the chill there is a marked decrease in the heat production which falls to within 20 to 38 per cent. of the average basal level. During the remaining periods it decreases slowly until it reaches the normal.

Heat Elimination: During the preliminary period the heat elimination, of course, equals heat production. During the prodromal period before the chill it shows little or no change. During the chill the heat elimination remains almost exactly the same as during the preliminary period. In spite of the enormous increase in heat production the amount of heat which leaves the surface of the body is almost exactly the same as before the chill. The blanching and coldness of the surface have not decreased the actual amount of heat eliminated. In the fourth period, of rising temperature after the chill, there is slight increase in the heat elimination, but, of course, the elimination still lags behind the production. In the fifth period, of continuous temperature, the heat elimination rises and begins to equal the heat production. In the sixth period, of falling temperature, the heat elimination is greatly increased.

Vaporization: The actual amount of heat lost through the vaporization of water through skin and lungs amounts to about 25 per cent. of the total heat *elimination* in the preliminary period. This percentage ratio remains fairly constant during all the periods. The actual number of calories lost by vaporization per hour changes very little during the first five periods, but rises abruptly during the last period. The patient then loses a large amount of heat during the vaporization of sweat.

Respiratory Quotient: During the prodromal period and chill, as a rule, the respiratory quotient is higher than after the chill, suggesting the rapid combustion of the glycogen stores during the violent muscular exercise.

(*j*) *Changes in Rectal Temperature and in Average Body Temperature.*—Maragliano,[17] in 1888, working on malaria patients with a Mosso plethysmograph, found that the volume of the arm decreased not only during the chill but before the chill, even before any chilly sensations had been felt. When the temperature began to fall the volume of the arm increased to a point far above its original level.

16. Lusk, G.: (Footnote 11) found a maximum increase of 180 per cent. in the heat production of a thin man during the violent shivering which followed immersion for nine minutes in water at a temperature of 10 C.

17. Maragliano: Das Verhalten der Blutgefässe im Fieber und bei Antipyrese. Ztschr. f. klin. Med., 1888, **14**, 309.

He concluded from his experiments that before the chill there is a contraction of the superficial blood vessels which precedes rise in temperature; that during the chill the blood vessels are still further contracted; and that they remain in that condition until the temperature begins to drop, when there is a marked dilatation. He found, also, that the dilatation of blood vessels precedes by a short interval the drop in temperature, but continues to increase during the rapid fall.

It may be that we have here in this curve a confirmation of these findings. Just as Maragliano found a contraction in superficial blood vessels before the rise in temperature, so we have found an increase in the temperature of the rectum before the chill. From these facts it might be assumed that the blood is driven from the surface to the inside of the body. Any extra heat which is produced during this period would, under these conditions, be stored in the interior of the body. The rectal temperature would therefore rise.

So, also, during the chill and in the hour following it the heat produced is stored more rapidly in the deeper organs of the body than near the surface.

When the rectal temperature begins to fall the average body temperature is still rising and probably continues to do so for a considerable period.

During the stage of falling temperature the heat which has been stored in the deeper structures is being given off rapidly to the rest of the body. The heat elimination has been raised to an abnormal level. But, with both radiation and conduction and water elimination increased, the skin and lung surfaces are not able to throw off the heat as rapidly as it is produced anew and delivered from the inside of the body.

This condition might be represented by the following simple diagram:

Interior of body ⇉ Periphery of body ⟶ Outside air.

The interior of the body is losing heat rapidly to the surface layers of the body, which are in turn losing heat less rapidly to the outside air. The mechanism of heat loss in the body has not yet recovered its function sufficiently to eliminate the heat which is already stored in the body as well as that which is being produced at the time.

It is interesting and important that, during this obvious attempt on the part of the body to rid itself of heat, there is no marked decrease in heat production. To be sure, there is a very gradual lowering of production to the normal level, but there apparently is no mechanism by which the heat production can be lowered abruptly. It may be that during the drop in temperature the heat production is proportional to the level of the temperature. During the end of the fall the heat pro-

duction is on a much lower level. The heat elimination is high. The inside of the body loses heat slowly; the periphery eliminates it rapidly. The mechanism of loss has by this time apparently recovered its complete function. This simply means that the heat which had accumulated at the surface of the body is now being eliminated. The inside of the body has lost to the periphery most of the heat which was stored within it during the period of high fever and is now near its normal level.

The results on the heat lost in vaporization show two things with distinctness. Formerly, the water elimination has been studied chiefly in patients without fever. Under these conditions, over any extended period, the heat production, as figured by the method of direct calorimetry, equals the heat elimination. Under these circumstances it does not matter whether the heat of vaporization be compared to the heat production or to the heat elimination. In fever observations, however, the heat elimination and heat production vary within wide limits. Thus, in the observations on malaria, comparison of the heat of vaporization with the heat production gives variations of 10 to 45 per cent. On the other hand, the comparison of the heat of vaporization with the heat eliminated shows variations within much narrower limits, 23 to 36 per cent. If the observation on the sweating patient, Sam F., be omitted the variation is between 23 and 31 per cent. It can therefore be said with some degree of certainty that heat of vaporization is a function of heat elimination and that it has no relationship to the heat produced.

In the rapid fall, with sweating, observed on Sam F., the percentage of heat of vaporization to total heat eliminated was even more increased above the usual level than is shown by the figures. It is certain in this case that not all of the water was removed by the ventilation of the chamber.

VI. SUMMARY AND CONCLUSIONS

Eleven calorimeter experiments have been made on patients in different periods of malarial fever, six of them being made during paroxysms. In all cases the methods of direct and indirect calorimetry were used. For the total series of experiments the calories, as measured by these two independent methods, agree within 0.08 per cent. of each other. In some of the individual observations the divergence between the two was as great as 7 per cent.; in some of the short periods the divergence was much greater.

The rectal temperature, which was measured every four minutes, showed typical malarial curves. The temperature curve of the deep parts of the body, as represented by the rectal region, does not indicate correctly the changes in the average body temperature. In a man

weighing 70 kg. about 15 kg. of tissue lie within 1 cm. of the surface. This subcutaneous layer may be warm or cold. When it is warm the blood from the interior of the body is cooled at the surface of the skin. When the subcutaneous layer is cold the largest mass of blood loses heat 1 to 3 cm. below the skin. A man producing a constant amount of heat every hour may eliminate from his surface exactly the same amount of heat with warm skin as with cool skin. A malarial patient may eliminate exactly the same amount of calories per hour before his chill as he does during the period of high temperature before the sweating begins. The changes in the average temperature of the body have been measured by a method based on the difference between the indirect and direct calorimetry during individual periods. This new method is possible only with a respiration calorimeter.

The periods of malarial paroxysms may be divided as follows:

1. *Preliminary.* Temperature constant; no change in metabolism.

2. *Prodromal.* Fifteen or twenty minutes before the chill there is a slight rise in rectal temperature.

3. *Chill.* Rectal temperature rises abruptly; the surface of the body becomes relatively and perhaps actually cooler; the average body temperature rises somewhat less abruptly than the rectal temperature.

4. *Rising Temperature After Chill.* Rectal temperature rises less rapidly than during chill; the surface becomes warmer.

5. *High, Continuous Temperature.* Rectal temperature is constant; surface temperature rises steadily; patient feels hot.

6. *Falling Temperature.* Rectal temperature falls much more gradually than it rises. The surface temperature at first may continue to rise, then to fall gradually for a period, and later to fall at about the same rate as the rectal temperature.

The *heat production* increases 100 to 200 per cent. during the chill; immediately after the chill it falls to within 20 to 38 per cent. of the average basal level; with the falling temperature the heat production drops to normal.

During the period before the chill with constant temperature the *heat elimination,* of course, equals the heat production. During the chill, in spite of the enormous increase in heat production, the heat elimination is the same as in the preliminary period. Almost all of the extra heat produced is stored in the body tissues.

In the fourth period of rising temperature after the chill there is a slight increase in heat elimination. In the fifth period of continuous temperature heat elimination begins to equal heat production. In the sixth period of falling temperature the heat elimination is greatly increased, chiefly by means of a large increase in the vaporization of water from the skin. Of the total calories produced in this period the

patient loses a much larger percentage than normal through vaporization. On the other hand, the percentage of calories lost through vaporization is not greatly increased in its relationship to the total heat elimination.

The respiratory quotients during the chill are higher than before or after the chill, suggesting the rapid combustion of glycogen stores during the violent muscular exercise of shivering.

CONCLUSIONS

In malarial paroxysms the increase in body temperature is due to an increasing heat production, which more than offsets a slightly increasing heat elimination.

The fall in body temperature is due to a greatly increased heat elimination with a heat production which is very slightly raised above the normal.

There is no indication of abnormal processes of metabolism in malarial fever except that the protein metabolism is somewhat increased.

In two observations on patients after treatment the basal heat production was normal.

During a malarial paroxysm the rectal temperature rises somewhat more rapidly than the average body temperature.

Except for the period of chill the level of the heat production is roughly proportional to the height of the body temperature.

During a malarial paroxysm the percentage of heat lost in vaporization of water bears a fairly constant relationship to the heat elimination but not to the heat production.

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A FURTHER STUDY OF ETHYLHYDROCUPREIN (OPTOCHIN) IN THE TREATMENT OF ACUTE LOBAR PNEUMONIA *

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In a previous communication[1] we reported on a series of thirty-two cases of lobar pneumonia due to pneumococci and treated with ethylhydrocuprein (optochin) hydrochlorid. By means of bactericidal tests of the patient's serum in vitro the absorption and elimination of the drug in these cases was studied. It was concluded that the hydrochlorid of the drug is rapidly absorbed from the gastro-intestinal tract into the circulating blood; that when an amount of the hydrochlorid represented by 0.024 to 0.026 gm. per kilogram of body weight of the patient is administered by mouth per twenty-four hours, the blood serum of the patient acquires the property of destroying pneumococci in the test tube; that the best way to insure the rapid production and maintenance of this bactericidal action in the blood is to divide the total amount of the drug in such a way that the first dose is relatively large and is followed at intervals of not more than three hours by smaller equal doses. For example, if the patient is of average size and is to receive 1.5 gm. in twenty-four hours, he is given a first dose of 0.45 gm., and this is followed by seven doses of 0.15 gm. each at regular intervals. It was further shown that during administration of the drug the pneumococci in the body may become "fast" or resistant to a considerable concentration of ethylhydrocuprein.

The purpose of the present paper is to present the data accumulated in an extension of the former work and to give the final results of two years' experience in the treatment of lobar pneumonia with ethylhydrocuprein. It may be stated that the experimental results obtained during the first year have been for the most part confirmed by the study of cases treated during the second year, and, in addition, some new facts have been ascertained which it is desirable to record. The experimental observations will be recorded first, and then the entire series of cases will be analyzed from a clinical standpoint.

The method of investigation described in the previous paper has

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1. Moore, H. F., and Chesney, A. M.: THE ARCHIVES INT. MED., 1917, **19,** 611.

again been used and has been found satisfactory. Briefly, this consisted in repeated determinations of the bactericidal power of the patient's serum at 37.5 C. for young (four to six hours) broth cultures of pneumococci. The strains used were stock strains and usually the type of pneumococcus employed for any case differed from that causing the infection. Almost all the patients admitted to the wards of the Hospital of The Rockefeller Institute from October, 1915, to May, 1917, suffering from lobar pneumonia due to pneumococci belonging to Groups II, III and IV, were treated with ethylhydrocuprein. Cases due to pneumococci of Group I did not receive ethylhydrocuprein, but were treated with specific immune serum. A limited number of patients infected with pneumococci of Groups II and III were treated with ethylhydrocuprein by mouth and in addition received type homologous immune serum intravenously, or concentrated "extract" of such serum subcutaneously or intravenously.

Of the forty-three patients treated with ethylhydrocuprein during the season 1916-1917, two received the base (optochin base) by mouth. Forty received the hydrochlorid by mouth and one patient received the hydrochlorid intramuscularly at first, and later by mouth. In Table 8 are presented the details as to dosage of the drug, clinical features and so forth, in these forty-three cases.

ADMINISTRATION OF ETHYLHYDROCUPREIN BASE BY MOUTH

The serum of the patients (Nos. 2786 and 2783) who received the base by mouth failed to show either bactericidal activity or power temporarily to inhibit the growth of pneumococci, although the amount of the drug given should have been sufficient to produce such a result if the base were as readily absorbable as the hydrochlorid. The difficulty of absorption from the gastro-intestinal tract is undoubtedly dependent on the fact that the drug in this form is very slightly soluble.

"FASTNESS" OF PNEUMOCOCCI TO ETHYLHYDROCUPREIN

In our former communication we reported one instance in which the infecting pneumococcus became "fast" to ethylhydrocuprein in the human body. A further example of this phenomenon has been observed in the present study. The details follow:

Hosp. No. 2825.—A street cleaner, aged 50; weight 69 kg.

Past History.—Unimportant.

Present Illness.—Cough for one day; chill and bloody expectoration on day of admission.

Status on Admission.—Patient dyspneic, cyanotic and decidedly ill; temperature 105.2 F.; pulse 120; respirations 32; dulness with suppression of breath sounds and numerous fine moist râles over left lower lobe. Leukocyte count 15,000. Sputum tenacious, hemorrhagic and containing Pneumococcus Type II. Blood culture showed Pneumococcus Type II, three colonies per cubic centimeter.

Course and Treatment.—Ethylhydrocuprein hydrochlorid started by mouth on second day of disease; schedule as follows in periods of twenty-four hours: first period, 1×0.6 gm. $+ 8 \times 0.15$ gm. (0.026 gm. per kilo of body weight per twenty-four hours); second period, 12×0.15 gm.; third period, 11×0.15 gm.; last period of eighteen hours, 7×0.15 gm. $+ 2 \times 0.1$ gm. Blood culture taken thirty-six hours after the commencement of the ethylhydrocuprein treatment showed twenty-four colonies of pneumococcus Type II per cubic centimeter of blood and the patient's general condition seemed worse at that time. On the following day the blood culture showed sixty colonies per cubic centimeter and on the last day of life blood culture showed 700 colonies of Pneumococcus Type II per cubic centimeter of blood. The patient became progressively worse and died on the fifth day after admission to the hospital. Judging from physical signs, spread of the infected area in the lungs did not take place. The total amount of ethylhydrocuprein given was 6.5 gm. No toxic symptoms referable to the drug were observed.

Samples of blood serum of this case were obtained before beginning treatment and eight times during the course of treatment; these samples were tested for pneumococcidal power against a four-hour-old culture of Pneumococcus Type II. Pneumococci grew unhindered in the specimen obtained before beginning treatment, but the tests of the specimens obtained later showed that the ethylhydrocuprein conferred the usual degree of pneumococcidal power on the serum as early as 5.5 hours after the first dose was administered and that this pneumococcidal power was maintained throughout the treatment.

The specimens of patient's serum showing pneumococcidal power were pooled and the effect of the pooled serum on two strains of pneumococci, obtained from the patient's blood, was studied; one of these strains was obtained from the patient's blood on admission to the hospital and before the ethylhydrocuprein treatment was started and the other strain from his blood two hours before death. The results are given in Table 1. The technic employed was that previously described,[1] using 0.001 c.c. of a four-hour-old broth culture to inoculate 3 c.c. of serum. The serum was previously heated at 56 C. for one half hour. The bacterial count of the plates is given in approximate figures.

TABLE 1.—Results of Ethylhydrocuprein Injections in Hospital No. 2825

Serum	Strains of Pneumococcus Obtained from Blood	Number of Colonies of Pneumococci per 0.5 C.c. of Serum When Plated*		
		Immediately	After 1½ Hours Incubation	After 24 Hours Incubation
Before ethylhydrocuprein was started	On admission (before ethylhydrocuprein)	400	2,000	Confluent
	Two hours before death	400	2,000	Confluent
Pooled specimens obtained during ethylhydrocuprein treatment	On admission (before ethylhydrocuprein)	400	300	0
	Two hours before death	400	2,000	Confluent

* Approximate counts.

From Table 1 it is seen that whereas the strain obtained from the patient's blood before the ethylhydrocuprein treatment was started was readily killed by the pooled serum obtained during the administration of the drug, the strain obtained from the blood towards the end

of the treatment and shortly before the death of the patient was completely insusceptible to any pneumococcidal action of the same serum.

The effect of various concentrations of ethylhydrocuprein hydrochlorid in broth on the growth of the different strains of pneumococci obtained from this patient was also studied. In each test 0.1 c.c. of an eighteen-hour broth culture of the given strain was inoculated into 5 c.c. of the broth containing the ethylhydrocuprein, mixed and incubated for forty-eight hours. The degree of growth, if any, was judged macroscopically. Plates were also poured and smears examined after incubation.

TABLE 2.—Growth in Broth Containing Various Dilutions of Ethylhydrocuprein Hydrochlorid of Strains of Pneumococcus Isolated from Blood of Hospital No. 2825

Designation of Strain	Isolated	Number of Colonies in 0.000001 C.c. of Culture Used in Tests
A	From blood on admission	85
B	From blood on second day of ethylhydrocuprein treatment	101
C	From blood on third day of ethylhydrocuprein treatment	162
D	From blood on fourth day of ethylhydrocuprein treatment (2 hrs. before death)	150

Tube*	Dilution of Ethylhydrocuprein in Broth	Strain			
		A	B	C	D
1	1: 100,000	0	0	0	0
2	1: 200,000	0	0	0	0
3	1: 400,000	0	0	0	0
4	1: 600,000	0	0	0	Growth
5	1: 800,000	0	0	0	Growth
6	1:1,000,000	0	0	Growth	Growth
7	1:1,200,000	0	Growth	0	Growth
Control broth without ethylhydrocuprein		Growth	Growth	Growth	Growth

* Tubes incubated forty-eight hours at 37.5 C.

The results given in Tables 1 and 2 show definitely that during the course of treatment in this case the pneumococci in the body become gradually resistant to the action of the ethylhydrocuprein. The observations made in this case, together with the similar one made by us previously, not only demonstrate that this phenomenon of bacterial "fastness" may occur, but indicate that its occurrence is not infrequent.

BACTERICIDAL ACTION OF PERICARDIAL FLUID

We have previously reported[1] that a pericardial exudate obtained post mortem from a patient who had received ethylhydrocuprein hydrochlorid by mouth for several days possessed bactericidal properties. In the present series of cases the same phenomenon was demonstrated in pericardial fluids obtained post mortem from four other cases (Nos. 2800, 2849, 2919 and 3031). In each case the pericardial fluid was allowed to clot and the supernatant fluid was then pipetted off and used for bactericidal tests. Details of the tests of the pericardial fluid of two of these cases follow (Tables 3 and 4).

TABLE 3.—Test of Bactericidal Power of Blood Serum and Pericardial Fluid from Hospital No. 2800

Tube Number	Blood Serum Obtained*	Number of Colonies of Pneumococci per 0.5 C.c. When Plated†		
		Immediately	After 1½ Hours Incubation	After 19¼ Hours Incubation
1	Before ethylhydrocuprein	801	2,960	Confluent
2	12 hours after first dose	268	54	0
3	35 hours after first dose	421	121	0
4	Pericardial fluid obtained post mortem*	301	345	1

* The pericardial fluid contained many pneumococci and it and the serum were heated at 56 C. for three-fourths hour before testing to destroy the contained pneumococci.
† Inoculation: 0.001 c.c. of a four-hour broth culture of Pneumococcus Type II.

TABLE 4.—Test of Bactericidal Power of Blood Serum and Pericardial Fluid from Hospital No. 2919

Tube Number	Blood Serum Obtained	Number of Colonies of Pneumococci per 0.5 C.c. When Plated*		
		Immediately	After 1½ Hours Incubation	After 24 Hours Incubation
1	Before ethylhydrocuprein	1,200	2,400	Confluent
2	13 hours after first dose	1,200	Complete inhibition†	0
3	24 hours after first dose	800	Complete inhibition	84
4	69 hours after first dose	1,200	Complete inhibition	0
5	97 hours after first dose	1,000	Complete inhibition	0
6	Pericardial fluid obtained post mortem	2,000	.....	26

* Inoculation: 0.001 c.c. of a four-hour broth culture of Pneumococcus Type I.
† By complete inhibition of growth is meant no increase in the number of colonies.

In Hosp. No. 2849 the pericardial fluid obtained post mortem showed pneumococcidal action, although the blood serum obtained during the ethylhydrocuprein treatment showed only temporary inhibition of growth.

These observations show that when ethylhydrocuprein hydrochlorid is given by mouth according to the system of dosage used by us, it may pass into a serous sac (pericardial fluid) in amounts sufficient to exert a pneumococcidal action.

TOXIC DISTURBANCES OF VISION

In one of the patients of our former series[1] the administration of ethylhydrocuprein hydrochlorid led to the production of alarming and severe retinitis, from which, however, the patient recovered. In the present series eight patients complained of amblyopia, mild in three cases, more severe in five. On discontinuing the ethylhydrocuprein, vision was restored in all those who survived the pneumonia, and in the two who died the vision was improved after the discontinuance of the drug. Some details of these eight cases follow:

Hosp. No. 2940.—Housewife, aged 31; weight 74.2 kg.

Past History.—Unimportant.

Present Illness.—Chill, fever and pain in chest six days before admission.

Status on Admission.—Temperature 104.2 F.; pulse 136; respirations 44. Consolidation of left upper lobe; leukocyte count 41,400. Sputum tenacious, gray and contained Pneumococcus Type IV. Patient quite ill. Blood culture positive (four colonies per cubic centimeter).

Course and Treatment.—Ethylhydrocuprein hydrochlorid was started by mouth on the day of admission (seventh day of the disease) on a basis of 0.0269 gm. per kilogram of body weight for the first twenty-four hours (1×0.5 gm. $+ 10 \times 0.15$ gm.). During the second twenty-four hours 0.15 gm. was given every two hours. One hour after the second of these latter doses the patient complained of slight blurring of vision; later the vision seemed normal and the hearing became slightly impaired; about the time of the seventh dose (second twenty-four hours) the patient complained of "waves" before her eyes and continuous ringing in the ears and one and one half hours after this dose she complaind that everything was "blurred and indistinct." The ethylhydrocuprein was therefore discontinued and after about eight hours the patient was seen by Dr. W. W. Weeks. His report on her visual condition stated that both disks were pale, that there were numerous small areas of edema scattered throughout the fundi, and that the patient could at this time count fingers at a distance of 6 feet. The patient died two days after the ethylhydrocuprein was stopped. The total amount of ethylhydrocuprein given was 3.05 gm.

The bactericidal test of the serum of this case showed that pneumococcidal action for a stock strain of Pneumococcus Type II was present in the serum ten hours after the administration of the first dose of ethylhydrocuprein (plate poured immediately after inoculation showed 800 colonies; plate poured after one and one-half hours incubation, 400; and plate poured after twenty-four hours incubation was sterile). Pneumococcidal action was also present in serum obtained twenty-three hours after the initial dose. The pneumococcidal action was no longer present in serum obtained shortly before death (ethylhydrocuprein having been discontinued for two days).

Hosp. No. 2885.—Woman, cook, aged 42; weight 49 kg.

Past History.—Unimportant.

Present Illness.—Headache, pain in chest and vomiting two days before admission, followed next day by chill; little cough or expectoration.

Status on Admission.—Temperature 102.8 C.; pulse 96; respirations 32. Involvement of right upper lobe posteriorly; leukocyte count 28,000; sputum slightly tenacious, not hemorrhagic, contained Pneumococcus Type II.

Course and Treatment.—Ethylhydrocuprein hydrochlorid was started by mouth on the day following admission, on a basis of 0.03 gm. per kilogram of body weight for the first twenty-four hours (1×0.45 gm. $+ 7 \times 0.15$ gm.); thereafter 0.15 gm. every three hours. After a total amount of 1.95 gm. had been given, the patient complained that she could not see; the administration of ethylhydrocuprein was thereupon discontinued. The pupils at this time were not dilated. About one hour later the patient could distinguish the outline of persons. Examination of the eyegrounds revealed no marked abnormalities. On the following day the vision was much improved and the eyes were examined by Dr. W. W. Weeks, who reported that there was indistinctness of both disk margins, the fundi were pale, the veins were somewhat dilated and tortuous, the arteries were of normal size, the vision was 20/20 in both eyes, and the fields were normal. About this time the patient could distinguish colors and said she could see quite well. This patient received, in addition to the ethylhydrocuprein, 770 c.c. of antipneumococcus serum, Type II, intravenously, in divided doses. The temperature reached normal on the eighth day of the disease. Pneumococcidal power for a stock strain of Pneumococcus Type I was present in the serum of this case within twelve hours after the initial dose of ethylhydrocuprein.

Hosp. No. 2870.—Housewife, aged 46; weight 56.9 kg.

Past History.—Two previous attacks of pneumonia.

Present Illness.—Three days before admission, chill, nausea, pain in chest, cough, blood-tinged sputum.

Status on Admission.—Consolidation of left lower lobe; well marked friction rub over entire precordial region; area of cardiac dulness not enlarged. Temperature 105.1; pulse 112; respirations 34. Sputum bright, rusty-red, mucopurulent and contained Pneumococcus Type II.

Course and Treatment.—Ethylhydrocuprein hydrochlorid was started by mouth the day after admission (fourth day of disease) on a basis of 0.0263 gm. per kilogram of body weight per twenty-four hours (1×0.45 gm. $+ 7 \times 0.15$ gm.; thereafter 0.15 gm. every two and a half hours). On the second day of treatment with ethylhydrocuprein the patient seemed slightly deaf and after 2.7 gm. of ethylhydrocuprein had been given, on being questioned, the patient said that she could not see. The pupils were widely dilated. The ethylhydrocuprein was thereupon discontinued. Three and a half hours later she said her sight was better, and in six hours after the last dose of ethylhydrocuprein she could distinguish objects. The next day her sight was considerably better and the pupils less dilated. The patient was seen by Dr. W. W. Weeks a few hours after the ethylhydrocuprein treatment had been discontinued. Dr. Weeks reported as follows: "Fingers can be seen 1 foot distant; visual fields moderately contracted; color not recognized; media clear; disks and fundi pale; veins engorged; arteries somewhat narrow." Dr. Weeks' report two days later was as follows: "vision 20/40 + with both eyes; visual fields contracted, especially on nasal side; red or green cannot be distinguished; disks pale, especially on temporal side; fundi not pale; condition of vessels same as on previous examination." Her vision gradually improved and was normal when she left the hospital. Pneumococcidal power for a stock strain of Pneumococcus Type I appeared in the blood serum of this patient within twenty-four hours of the administration of the first dose of ethylhydrocuprein.

Hosp. No. 2812.—Woman, aged 74; weight 58.5 kg.

Past History.—Unimportant.

Present Illness.—The patient said that she had not felt well for several days before admission. On the morning of the day before admission she felt very ill and had a temperature of 105 C., cough, blood-tinged sputum and pain in chest.

Status on Admission.—Temperature 104.1 F.; pulse 94; respirations 40. Involvement of the left lower lobe; emphysema present; sputum tenacious, rusty and yielded on passage through a mouse, *Pneumococcus mucosus* (Type III).

Course and Treatment.—Ethylhydrocuprein hydrochlorid was started by mouth on the day of admission, on a basis of 0.03 gm. per kilogram of body weight per twenty-four hours (1×0.6 gm. $+ 8 \times 0.15$ gm.; thereafter 0.15 gm. every two hours). After 0.9 gm. of the ethylhydrocuprein had been given the patient complained of hearing roaring noises, and after 3 gm. of ethylhydrocuprein she complained that she could not see. The ethylhydrocuprein was then discontinued. In six hours, however, she was able to count fingers and the next day her eyesight was much improved. Eight hours after the patient complained of loss of vision she was seen by Dr. W. W. Weeks, who reported that there was little change in the eyegrounds. The patient died on the fifth day after admission to the hospital.

Pneumococcidal action for a stock strain of Pneumococcus Type II appeared in this patient's serum six hours after the initial dose of ethylhydrocuprein.

Hosp. No. 3015.—Engineer, aged 47; weight 75.8 kg.

Past History.—Unimportant.

Present Illness.—Chill, vomiting, headache, and pain in chest thirty-six hours before admission.

Status on Admission.—Temperature 104.6 F.; pulse 128; respirations 34. Consolidation of left lower lobe. Sputum obtained on admission was tenacious, frothy, rusty and contained *Pneumococcus mucosus* (Type III). Urine gave a definite precipitin reaction with antipneumococcus serum Type III.

Course and Treatment.—Ethylhydrocuprein hydrochlorid was started by mouth eleven and one-third hours after admission to the hospital on a basis of 0.034 gm. per kilogram of body weight per twenty-four hours (1×0.6 gm. $+ 10 \times 0.2$ gm.). After 2.4 gm. had been given the patient complained that he could not see distinctly and the ethylhydrocuprein was discontinued. At that time he could not see objects 1½ feet from his eyes, but could distinguish direct light from darkness; the pupils were somewhat dilated and did not react to light; the veins were engorged and the eyegrounds somewhat pale. He had been questioned two and three-quarters hours previously as to whether he could see well, and replied that his vision was as good as normal; at that time he was partially deaf. Twelve hours after the complaint that vision was impaired, the patient could read a watch at 2 feet and a ward clock at about 20 feet, and said he could see well, but that objects were a little blurred. Later that day he was examined by Dr. W. W. Weeks, whose report is abstracted as follows: "Vision 20/40 + in both eyes; arteries injected; veins tortuous and engorged; left disk distinctly pale, especially the temporal half; visual fields restricted in both eyes, 10 degrees in right, and 20 degrees in left; color is recognized by left eye but not by right." Three days later Dr. Weeks reported as follows: "Pupils equal, contracted, reacted readily to light during accommodation and on convergence; vision without correction: O. D. 20/40 +, O. S. 20/210 with + 1.25 O. D. 20/20, with + 1.50 O. S. 20/20; concentric contraction for form, more in left eye; media clear; retina somewhat hazy near disks, so as to make disk margin a little indistinct; vessels normal except arteries of left eye which were somewhat smaller and more tortuous than those of right."

The temperature reached normal on the night of the day following admission to the hospital, but rose again thirty-six hours afterward and remained elevated for four days, although the pulse and respiration rates did not increase and the patient felt quite comfortable. The patient recovered.

The test of blood serum in this case showed pneumococcidal action for a stock strain of Pneumococcus Type II.

Hosp. No. 2972.—Man; complained of slight transient blurring of vision at times after the temperature had become normal; ethylhydrocuprein was discontinued (see Table 8 for details of the case).

Hosp. No. 2837.—Man; complained of transient deafness; transient dimness of vision occurred after the temperature had become normal; ethylhydrocuprein was discontinued (see details of case in Table 8); the patient had an abrasion of the cornea and there was some inflammation of the right cornea on the morning that he complained of dimness of vision.

Hosp. No. 2911.—Woman; complained of dimness of vision and slight partial deafness after she had received 2.95 gm. of ethylhydrocuprein hydrochlorid by mouth; both passed off after ethylhydrocuprein had been discontinued (for details of the case see Table 8).

In our entire series of seventy-five cases, nine, or 12 per cent. of the patients, showed some degree of amblyopia. Of all the patients treated with ethylhydrocuprein which are recorded in the literature, between 4 and 5 per cent. suffered from amblyopia, and in two of these the impairment of vision was more or less permanent (Oliver[2] and Lorant[3]). In these latter cases, however, the dosage of the drug seems to have been excessive.

RELATION OF NUMBER OF PNEUMOCOCCI TO CONCENTRATION OF ETHYLHYDROCUPREIN IN BACTERICIDAL TESTS

In the technic employed in the bactericidal tests we have used a fairly constant and rather small number of pneumococci per cubic centimeter. It seemed advisable to determine whether or not the degree of bactericidal action would be the same when a larger number of pneumococci are employed. Specimens of broth containing a small amount of ethylhydrocuprein in solution were therefore inoculated separately with two different amounts of the same culture and incubated at 37.5 C. At frequent intervals plates were prepared and counts were made of the colonies which developed after incubation.

Experiment 1.—Fifty c.c. of bouillon containing ethylhydrocuprein hydrochlorid 1 to 1,000,000 were inoculated with 0.01 c.c. of a twenty-four hour culture of Pneumococcus Type II and a like amount of the same bouillon with 1 c.c. of the same culture. Similarly, two flasks containing 50 c.c. of broth each but without ethylhydrocuprein were inoculated with 0.01 c.c. and 1 c.c. of the culture, respectively, as controls. All four cultures were incubated at 37.5 C. and bacterial counts were made at frequent intervals by making suitable dilutions and pouring plates with 20 c.c. dextrose agar. The results of the experiment are given in Table 5.

2. Oliver, G. H.: Brit. Med. Jour. 1916, **1**, 580.
3. Lorant, L.: Deutsch. med. Wchnschr., 1916, **42**, 1355.

TABLE 5.—Number of Pneumococci in Relation to Action of Ethylhydrocuprein Hydrochlorid

Incubation		Broth without Ethylhydrocuprein Inoculation 0.01 C.c.		Broth with Ethylhydrocuprein 1:1,000,000 Inoculation 0.01 C.c.		Broth without Ethylhydrocuprein Inoculation 1.0 C.c.		Broth with Ethylhydrocuprein 1:1,000,000 Inoculation 1.0 C.c.	
Hours	Minutes	No. of Viable Pneumococci per 0.5 C.c.	Log.	No. of Viable Pneumococci per 0.5 C.c.	Log.	No. of Viable Pneumococci per 0.5 C.c.	Log.	No. of Viable Pneumococci per 0.5 C.c.	Log.
0	0	28,000	4.447	19,050	4.379	2,455,000	6.390	2,450,000	6.389
2	0	19,300	4.285	11,450	4.05	2,870,000	6.457	1,685,000	6.226
4	0	9,750	3.989	475	2.676	7,050,000	6.848	1,005,000	6.002
5	30	11,450	4.058	37	1.568	22,500,000	7.352	625,000	5.795
7	0	11,850	4.073	2	0.047	26,500,000	7.423	370,000	5.568
9	0	34,500	4.537	0	0	49,500,000	7.694	210,000	5.322
11	35	625,000	5.795	0	0	67,500,000	7.829	100,000	5.000
25	..	49,500,000	7.694	0	0	55,500,000	7.744	240,000	5.380
48	..	.....	.....	.....	.....	51,000,000	7.707	45,000,000	7.653

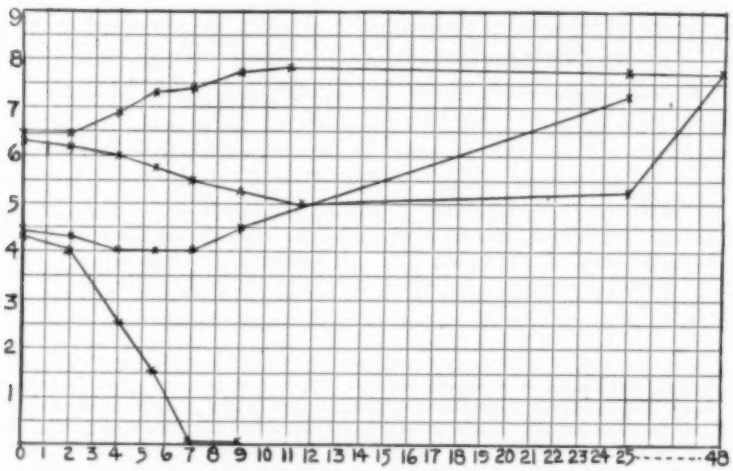

Diagram illustrating Experiment 1. Time in hours as abscissae at bottom, and at left logarithms of numbers of viable pneumococci per 0.5 c.c. as ordinates. A, Broth without ethylhydrocuprein. Inoculation 0.01 c.c. B, Broth with ethylhydrocuprein (1:1,000,000). Inoculation 0.01 c.c. C, Broth without ethylhydrocuprein. Inoculation 1 c.c. D, Broth with ethylhydrocuprein 1:1,000,000. Inoculation 1 c.c.

This experiment shows that while 19,050 pneumococci were all killed in nine hours in broth containing ethylhydrocuprein 1 to 1,000,000, 2,450,000 pneumococci were reduced to 100,000 in eleven hours and thirty-five minutes, but the surviving pneumococci were able to grow and in forty-eight hours multiplied approximately to the same extent (45,000,000) as in the control broth culture. The logarithms of the bacterial count are shown in Table 5. The results of Experiment 1 are shown graphically by plotting curves (Fig. 1), employing the time in hours as abscissa and the logarithms of the numbers of viable pneumococci per 0.5 c.c. of culture as ordinates.

PENETRATION OF ETHYLHYDROCUPREIN INTO FIBRINOUS EXUDATES

In the course of our clinical and experimental observations on ethylhydrocuprein, we have received the impression that the drug does not readily penetrate the alveolar exudate. The concentration of ethylhydrocuprein in the blood of patients receiving the drug, according to the dosage most commonly used by us, is about 1 in 500,000, as judged by the pneumococcidal action of the serum in the test tube. If ethylhydrocuprein in this concentration were to thoroughly penetrate the alveolar exudate, it should destroy the pneumococci present therein, and one might therefore anticipate a shortening of the duration of the disease. Since the duration of the disease is not shortened the following experiment was devised to study the power of ethylhydrocuprein to penetrate a fibrinous clot.

Experiment 2.—Fifty c.c. of normal rabbit blood were drawn into 1 c.c. of sterile 20 per cent. sodium citrate solution ($2Na_3C_6H_5O_7.H_2O$) and centrifugalized. The supernatant plasma was drawn off and inoculated with a stock strain of Pneumococcus Type II (0.0001 c.c. of an eighteen-hour culture to 1 c.c. plasma) and 1.1 c.c. of sterile 9.5 per cent. calcium chlorid solution was then added and thoroughly mixed. The mixture was transferred to sterile cotton-plugged glass tubes 0.9 cm. in diameter, 2 c.c. being placed in each tube. A firm clot formed at the bottom of each tube in about one to one and a half hours at room temperature. To one of the tubes 1.6 c.c. of 0.85 per cent. saline solution was then added, covering the clot, and, in a similar manner, to each of the remaining tubes there was added 1.6 c.c. of saline solution containing a given concentration of ethylhydrocuprein hydrochlorid. The tubes were kept at room temperature for one-half hour, then incubated for twenty hours at 37.5 C., and examined. The number of organisms used for the incubation had been so chosen that the control tube which contained no ethylhydrocuprein showed small discrete colonies uniformly "peppering" the clot. In the tubes containing the more concentrated ethylhydrocuprein solutions no growth occurred in the upper portions of the clot. This was interpreted as due to the effect of the ethylhydrocuprein penetrating the clot in effective concentration to the depth of the zone of inhibition. The depth of the zone of inhibition bore a definite relationship to the concentration of the ethylhydrocuprein in the supernatant fluid. The zones of inhibition in the various tubes were measured and the results are shown in Table 6.

TABLE 6.—Inhibition by Ethylhydrocuprein of Growth of Pneumococci in Fibrinous Clots

Dilution of Ethylhydrocuprein	Depth in Mm. of Zones of Inhibition of Growth	Dilution of Ethylhydrocuprein	Depth in Mm. of Zones of Inhibition of Growth
1:100	10	1:10,000	5.5
1:500	9.5	1:50,000	3.5
1:1,000	9.0	1:100,000	2.5
1:5,000	6.0	1:200,000	1.75
1:10,000	5.5	1:300,000	1.25
1:50,000	3.5	1:400,000	1.0
1:100,000	2.5	1:500,000	0.0
1:500,000	0.0	1:600,000	0.0
1:1,000,000	0.0		
Control with saline	0.0	Control with saline	0.0

These experiments demonstrate that optochin, in the concentrations attainable with any degree of safety in the blood plasma of patients (about 1 in 500,000), possesses little power to penetrate a fibrinous clot. It is, therefore, possible that pneumococci in the interior of a pneumonic exudate may escape the action of ethylhydrocuprein, even though the drug be present in the blood stream in considerable concentration.

CLINICAL OBSERVATIONS

In discussing the effect of ethylhydrocuprein on the clinical course of the disease, the patients treated during the two years will be considered as one series. As previously, they will be analyzed from the standpoint of (*a*) the duration of the disease, (*b*) the occurrence of "spread" of involvement of lobes hitherto unaffected, (*c*) the effect of treatment on pneumococcemia and (*d*) mortality with reference to the immunologic classification of the infecting strain of pneumococcus.

(*a*). *Effect on Duration of Disease.*—Using the occurrence of a rectal temperature below 100 F. as a criterion for the termination of the acute attack, an arbitrary but serviceable standard, the average duration of the disease in all the recovered patients was eight days. No marked shortening, therefore, of the course of the disease can be said to have occurred in our series.

(*b*). *Occurrence of "Spread" of Pulmonary Lesion.*—Of the 75 cases, 20, or 26.6 per cent., showed a "spread" during treatment. In 14 of these 20 cases the serum of the patient possessed bactericidal power at the time the spread took place; this may be taken as presump-

tive evidence that the drug, although circulating in the blood in amounts sufficient to destroy pneumococci, does not pass into the alveolar spaces or exudate in amounts sufficient to destroy the bacteria there or inhibit their growth to any marked extent.

(*c*). *Pneumococcemia.*—In 23, or 30.6 per cent. of the 75 cases, viable pneumococci were present in the circulating blood at some time during the course of the disease. In many instances there were only a few pneumococci present, whereas in others there was a comparatively heavy blood infection. In 19 of the 23 cases the blood culture was positive before ethylhydrocuprein treatment was begun; in 4 of these the blood culture became negative during treatment with ethylhydrocuprein alone; 9 of the cases showed a progressively increasing number of micro-organisms while under treatment, and the remaining 6 died without subsequent blood cultures having been made. When in addition to ethylhydrocuprein immune serum was used in treatment, the number of pneumococci in the circulating blood was always reduced after the first administration of serum, but in one instance the number of micro-organisms later increased even though the patient received both ethylhydrocuprein and serum. In 4 instances the blood culture was negative at the time the ethylhydrocuprein treatment was instituted, but became positive later. From these results it may be stated that no marked beneficial effect of ethylhydrocuprein treatment was observed on the pneumococcemia of lobar pneumonia.

(*d*). *Mortality.*—Of the 75 patients treated, 28 died—a mortality rate of 37.3 per cent. Some of the patients were inadequately treated, judging from the production of pneumococcidal power in the serum. Moreover, in several cases treatment was instituted less than twenty-four hours before death and at a time when the patients were critically ill; the duration of administration of the drug in these cases was, therefore, too short to be of any value, considering the time required for its absorption and action. If all the patients who are known to have been inadequately treated, regardless of whether they died or recovered, be excluded, and also those cases in which treatment was instituted within twenty-four hours of death, or in which crisis occurred before the serum acquired bactericidal power, there would remain 51 cases, of whom 16 patients died — a mortality of 31.3 per cent. Of the 24 cases excluded from consideration 12 patients died and 12 recovered. The mortality rate in the 51 adequately treated patients, 31.3 per cent., does not show any considerable therapeutic effect from the use of the drug.

It should be stated that the series probably represents a group of very severe cases, for it is composed for the most part of cases due to infection with pneumococci of Types II and III, which we know are

the types responsible for the highest mortality rates. When the expected mortality in each of these groups (untreated) is compared with that actually encountered in the patients treated with ethylhydrocuprein, we find some reduction of mortality rate in the cases infected with pneumococci belonging to Group III, and no reduction in the cases due to pneumococci of Groups II and IV. This will be seen from a study of Table 7, in which the cases are arranged according to the type of infecting pneumococcus and in which the mortality obtained in the various groups in our series is contrasted with that obtained in a large series of patients not treated specifically and observed in different clinics.[1] One is forced to conclude from these figures that treatment with ethylhydrocuprein hydrochlorid failed to cause any reduction in the general mortality rate.

TABLE 7.—MORTALITY AMONG PATIENTS TREATED WITH ETHYLHYDROCUPREIN HYDROCHLORID COMPARED WITH THAT OF PATIENTS NOT SPECIALLY TREATED

Pneumococcus Type	Number of Patients Treated with Ethylhydrocuprein	Recovered	Died	Mortality Rate Per cent.	Average Mortality Rate in Patients Not Specifically Treated
II	27	19	8	29.6	28
III	17	10	7	41.1	56
IV	6	5	1	16.6	16
Unclassified	1	1	0	0	..
	51	35	16	31.3	About 26

Of the 75 patients treated with ethylhydrocuprein, 14 in which the disease was caused by pneumococci belonging to Group II were treated, in addition, with the type homologous antipneumococcus serum. This agent was used in the form of the whole serum intravenously or the concentrated "extract"[4] of the serum subcutaneously and intravenously. Of these 14 patients 10 recovered and 4 died, giving a mortality rate of 28.5 per cent., so that no reduction in the mortality rate was observed in cases of lobar pneumonia due to Pneumococcus Type II as a result of treatment with ethylhydrocuprein and antipneumococcus serum. The number of patients treated with serum and ethylhydrocuprein is too small, however, to permit of final conclusions on this point.

DISCUSSION

The experimental studies which we have here discussed and those previously reported show that ethylhydrocuprein hydrochlorid fulfills at least some of the requirements of a chemotherapeutic agent in lobar pneumonia. Even in high dilutions it kills the pneumococcus in

4. Chickering, H. T.: Jour. Exper. Med., 1915, **22**, 248.

the presence of body fluids. It is capable of being absorbed from the gastro-intestinal tract, and when injected into the muscles (Case 2947, Table 8) may pass into the blood stream. Moreover, when a sufficient amount is administered by mouth, represented by 0.024 to 0.028 gm. per kilogram of body weight per twenty-four hours, the blood serum becomes pneumococcidal in vitro, and furthermore, when such a condition obtains in the blood, the pericardial fluid also becomes pneumococcidal. The amount of the drug which it is necessary to administer in order to achieve this result, however, cannot always be given with safety to the patient, for in one instance in our series of cases total blindness lasting six days resulted, and in eight other instances there occurred visual symptoms of sufficient gravity to make the discontinuance of the drug necessary. A study of Table 8 would seem to suggest that where there is a comparatively heavy septicemia (for example, 100 colonies per 1 c.c. or over) a dosage of ethylhydrocuprein represented by 0.026 gm. per kilogram of body weight per twenty-four hours may be insufficient to produce pneumococcidal actions in the blood serum. It is possible that in such instances the drug may be fixed by the circulating pneumococci (Case Nos. 2845 and 2892). In only one case (No. 2822) which received 0.026 gm. of the hydrochlorid of the drug, or over, per kilogram of body weight per twenty-four hours in suitably divided doses did pneumococcidal action fail to appear in the serum.

When the cases in our series are analyzed from the standpoint of the effect of the drug on the duration of the disease, on the occurrence of "spread" of the lesion to previously uninvolved lobes of the lung, on the pneumococcemia and on the mortality rate, the results do not afford much support for the routine use of this drug in the treatment of acute lobar pneumonia.

It seems to us that the main reason why ethylhydrocuprein has not produced more striking results in the treatment of lobar pneumonia is because the toxicity of the drug is such as to keep the limits of dosage below the limits of effectiveness. If larger doses could be safely employed, it would be possible greatly to increase the amount of ethylhydrocuprein circulating in the blood. This would in turn undoubtedly increase the rate and degree of the resulting pneumococcidal action in the blood and might conceivably lead to a greater penetration of the drug into the consolidated portions of the lung. Our tests show that ethylhydrocuprein may be administered to patients in amounts sufficient to cause the serum to acquire pneumococcidal power. With the dosage that may be safely employed, however, the serum exhibits its bactericidal activity only at a slow rate, considerable time being required. Because of this fact the pneumococci in the body may be exposed for considerable time to concentrations of the drug insufficient to cause their destruction, particularly when they are protected by the

TABLE 8.—SUMMARY

Hospital Case Number	Age	Weight, Kg.	Type of Infecting Pneumococcus	Day of Disease When Ethylhydrocuprein Treatment Was Begun	Lung Involvement on Admission	Blood Culture Before Treatment	Method of Dosage of Ethylhydrocuprein in Periods of 24 Hrs.* Gm.	Amount of Ethylhydrocuprein per Kilogram of Body Weight per 24 Hrs. Gm.	Total Amount of Ethylhydrocuprein in Grams	Duration of Treatment in Days
2750	27	55.8	II	3	L. L.	Sterile	0.45+7×0.15; 10×0.15	0.0269	5.7	4
2754	13	39.6	II	8	L. U. L. L.	.....	0.3+7×0.1; 10×0.1	0.0252	2.3	2¼
2782	48	62.3	III	7	R. U. R. L.	Positive; 225 col. per 1.0 c.c.	0.45+0.25+0.3; 2×0.15; base	.....	1.3	½
2786	34	42.7	II	8	R. U.	Sterile	0.3+0.2+7×0.10; 10×0.1; base	0.028	2.2	2
2797	67	78.2	III	2	R. U.	Sterile	0.6+2×0.25+6×0.15; 11×0.2	0.023	10.8	3
2800	42	78.9	III	4	L. L.	Sterile	0.6+0.3+8×0.2; 1.65 in 2nd 24 hrs.	0.03	4.15	2
2812	74	58.5	III	2	L. L.	Sterile	0.6+8×0.15; 12×0.15	0.03	3.0	2
2822	13	37.2	II	6	L. L.	Sterile	0.2+7×0.1; 9×0.1; 10×0.1	0.0241	3.6	4
2762	23	70.0	III	3	R. L.	Sterile	0.45+8×0.15; 3×0.5 per rectum	0.0235	3.15	2
2825	50	69.0	II	2	L. L.	Positive; 3 col. per 1 c.c.	0.6+8×0.15; 12×0.15	0.026	6.5	4
2827	29	52.6	II	3	L. L. L. U.	Positive; 2,000 cols. per 1 c.c.	0.45+2×0.15	0.0285	0.75	¼
2831	35	57.0	II	5	L. L.	Sterile	0.45+7×0.15; 10×0.15	0.0263	1.65	1
2834	28	58.4	II	5	L. L.	Sterile; 16 hrs. after ethylhydrocuprein positive—12 col. per 1 c.c.	0.5+0.2+6×0.15; 9×0.15; 10×0.15	0.0274	9.05	6
2837	48	46.0	III	2	R. L.	Sterile	0.45+7×0.15; 7×0.2	0.0326	2.9	2

* Given by mouth unless otherwise stated; hydrochlorid used unless otherwise stated.

OF CASES

Time of Appearance of Bactericidal Action in Serum after Initial Dose of Ethylhydrocuprein	Duration of Disease in Days	Toxic Symptoms Referable to Ethylhydrocuprein	Complications	Occurrence of "Spread" During Treatment	Result	Remarks
Not studied	7	None	None	None	Recovered	
Not studied	10	None	Pericarditis	None	Death	
No B. A.	7	None	Pericarditis	None	Death	Blood culture 8 hours after treatment showed innumerable colonies per 0.5 c.c. blood
No B. A.	9	None	None	None	Recovered	
20 Hrs.	7	None	None	Spread to right lower	Death	
11 Hrs.	6	None	None	None	Death	Blood culture 24 hours after treatment was begun showed 23 colonies per 1.0 c.c. blood; pericardial fluid showed bactericidal action
6 Hrs.	6	Temporary blindness, deafness and tinnitus	None	None	Death	Ethylhydrocuprein discontinued on account of eye symptoms
No B. A.; slight temporary inhibition	10	None	None	None	Recovered	
5 Hrs.	5	Persistent vomiting; partial deafness	None	None	Recovered	
5.5 Hrs.	5	None	None	None	Death	Progressive increase in septicemia during treatment. "Fast" strains recovered from blood; antipneumococcus serum 85 c.c. intravenously on last day
Not studied	4	None	None	None	Death	
11 Hrs.	6	None	None	None	Recovery	75 c.c. antipneumococcus serum intravenously
11 Hrs.	11	None	None	Spread to right lower	Death	Active maniacal delirium. Blood culture positive 16 hours after ethylhydrocuprein was started; 1,015 c.c. antipneumococcus serum intravenously
Within 24 Hrs.	4	Transient deafness; slight dimness of vision after Temp. had become normal	None	None	Recovery	Ethylhydrocuprein discontinued after temp. became normal on account of eye symptoms. Eye grounds apparently normal on ophthalmoscopic examination at time patient complained of dimness of vision

TABLE 8.—SUMMARY

Hospital Case Number	Age	Weight, Kg.	Type of Infecting Pneumococcus	Day of Disease When Ethylhydrocuprein Treatment Was Begun	Lung Involvement on Admission	Blood Culture Before Treatment	Method of Dosage of Ethylhydrocuprein in Periods of 24 Hrs.* Gm.	Amount of Ethylhydrocuprein per Kilogram of Body Weight per 24 Hrs. Gm.	Total Amount of Ethylhydrocuprein in Grams	Duration of Treatment in Days
2838	45	48.8	III	4	R. L.	Sterile	0.5+5×0.2; 8×0.2; 6×0.25	0.0307	7.6	5
2845	46	40.4	II	5	R. U.	Positive; 1,000 col. per 1 c.c.	0.5+4×0.25; 6×0.25	0.0371	2.0	1½
2849	42	94 4	III	4	R. U.	Positive; 1 col. in 3 c.c.	0.5+4×0.25; 6×0.25	0.0158	2.0	1½
2865	23	60.4	II	4	L. U.	Sterile	0.45+7×0.15; 10×0.15	0.0248	5.25	4
2869	31	66.6	II	2	R. L.	Positive; 400 col. per 1 c.c.	0.45+7×0.15; 10×0.15	0.0225	5.3	4
2870	46	56.8	II	4	L. L.	Sterile	0.45+7×0.15; 8×0.15	0.0263	2.7	2
2879	26	79.0	II	3	L. L.	Positive	0.5+9×0.15; 10×0.15	0.0234	3.45	2
2885	42	49.0	II	3	R. U.	Sterile	0.45+7×0.15	0.0300	1.95	1½
2886	21	59.8	II	4	L. U.	Sterile	0.45+7×0.15; 11×0.15	0.0250	5.25	4
2892	41	59.0	II	5	R. U.	Positive; 100 col. per 1 c.c.	0.45+7×0.15; 10×0.15	0.0254	2.25	1½
2890	28	35.5	II	4	R. L.	Positive; 3 col. per 1 c.c.	0.45+8×0.15; 10×0.15	0.0464	5.4	4
2897	34	59.0	II	4	L. U.	Sterile	0.45+7×0.15; 10×0.15	0.0254	6.0	4
2898	32	53.2	III	5	L. L.	Positive; 120 col. per 1 c.c.	0.45+7×0.15; 10×0.15	0.0283	3.75	3
2911	50	74.2	III	3	R. U.	Sterile	0.45+7×0.15+1×0.1; at rate of 10×0.15 thereafter	0.021	2.95	2

* Given by mouth unless otherwise stated; hydrochlorid used unless otherwise stated.

OF CASES

Time of Appearance of Bactericidal Action in Serum after Initial Dose of Ethylhydrocuprein	Duration of Disease in Days	Toxic Symptoms Referable to Ethylhydrocuprein	Complications	Occurrence of "Spread" During Treatment	Result	Remarks
Within 24 Hrs.	9	None	None	None	Recovery	
No B. A.; temporary inhibition only	7	None	None	None	Death	Pericardial fluid showed bactericidal action; 300 c.c. antipneumococcus serum intravenously
No B. A.	5	None	None	None	Death	Blood culture before death, 1,000 colonies per 1.0 c.c. blood
Within 24 Hrs.	7	None	None	None	Recovery	
No B. A.	6	None	None	Spread to right upper	Death	Blood culture constantly positive; 10,000 colonies per 1.0 c.c. just before death; 580 c.c. antipneumococcus serum intravenously
Within 24 Hrs.	9	Transient amblyopia and deafness	None	None	Recovery	Ethylhydrocuprein discontinued on onset of amblyopia
No B. A.; slight temporary inhibition	5	None	None	None	Recovery	Blood culture became negative during treatment; 440 c.c. antipneumococcus serum intravenously
Within 12 Hrs.	8	Transient amaurosis and tinnitus	None	None	Recovery	Ethylhydrocuprein discontinued on account of eye symptoms; 770 c.c. antipneumococcus serum intravenoutly; delayed resolution
Within 12 Hrs.	8	Slight deafness	None	None	Recovery	
No B. A.; slight temporary inhibition	7	None	None	Spread to right lower	Death	Blood culture before death, 600 colonies per 1.0 c.c. blood
Within 24 Hrs.	8	None	Pneumococcal meningitis	Spread to left lower	Death	Blood culture 18 hours after ethylhydrocuprein was commenced showed 14 colonies per 1 c.c.; 1,360 c.c. serum intravenously; 80 c.c. serum intraspinously; 30 c.c. concentrated serum subcutaneously
Within 12 Hrs.	8	None	None	None	Recovery	380 c.c. serum intravenously; 74 c.c. conc. serum subcutaneously
Within 24 Hrs.	8	None	None	Spread to right lower	Death	
Complete inhibition	8	Temporary blurring of vision and temporary partial deafness	None	None	Recovery	Ethylhydrocuprein discontinued on account of eye symptoms

TABLE 8.—SUMMARY

Hospital Case Number	Age	Weight, Kg.	Type of Infecting Pneumococcus	Day of Disease When Ethylhydrocuprein Treatment Was Begun	Lung Involvement on Admission	Blood Culture Before Treatment	Method of Dosage of Ethylhydrocuprein in Periods of 24 Hrs.* Gm.	Amount of Ethylhydrocuprein per Kilogram of Body Weight per 24 Hrs. Gm.	Total Amount of Ethylhydrocuprein in Grams	Duration of Treatment in Days
2919	35	49.8	III	4	L. L.	Sterile	0.45+7×0.15; 10×0.15	0.03	6.30	4
2922	30	58.0	II	4	L. L.	Sterile	0.45+7×0.15; 10×0.15	0.0258	11.1	8
2926	19	46.0	II	5	R. U.	Sterile	0.45+5×0.15; 7×0.15	0.026	3.0	2½
2927	22	56.4	III	3	R. L.	Sterile	0.45+7×0.15; 8×0.15	0.0265	4.95	4
2940	31	74.2	IV	7	L. U.	Positive; 4 col. per 1 c.c.	0.5+10×0.15; 12×0.15	0.0269	3.05	2
2943	22	44.4	II	5	R. L.	Sterile	0.4+8×0.1; 12×0.1	0.027	4.9	4
2946	28	62.6	II	4	R. U.	Sterile	0.5+8×0.15; 12×0.15	0.0271	12.8	8
2947	51	70.4	III	6	R. L.	Positive	0.7 intramuscularly + single dose of 1.8 gm. intramuscularly	0.0355	9.9	5
2973	40	50.2	III	3	R. U.	Sterile	0.45+7×0.15; 10×0.15	0.0375	3.15	3
2972	19	55.6	II	1	L. L.	Sterile	0.45+7×0.15; 10×0.15	0.0269	6.55	4½
2991	49	71.2	II	2	R. L.	Sterile	0.45+9×0.15; 6×0.3	0.025	14.4	8
3006	35	46.8	II	3	R. L.	Sterile	0.45+7×0.15; 10×0.15	0.032	7.8	5
3015	47	75.8	III	3	L. L.	Sterile	0.6+10×0.2	0.034	2.2	1
2868	20	57.0	II	5	R. L.	Sterile	0.45+7×0.15	0.0263	1.5	1
3031	38	57.0	II	3	L. L.	Positive; 80 col. per 1 c.c.	0.45+8×0.15; 9×0.15; 10×0.15	0.028	6.3	5½

* Given by mouth unless otherwise stated; hydrochlorid used unless otherwise stated.

OF CASES

Time of Appearance of Bactericidal Action in Serum after Initial Dose of Ethylhydrocuprein	Duration of Disease in Days	Toxic Symptoms Referable to Ethylhydrocuprein	Complications	Occurrence of "Spread" During Treatment	Result	Remarks
Within 12 Hrs.	9	None	None	Spread	Death	Pericardial fluid showed bactericidal action; blood culture sterile post-mortem
12 Hrs.	14	None	None	Spread to left upper	Recovery	197 c.c. conc. serum subcutaneously
12 Hrs.	8	Slight deafness	None	None	Recovery	
12 Hrs.	6	Temporary deafness	None	Spread to left upper	Recovery	
10 Hrs.	11	Dimness of vision and tinnitus	None	.....	Death	Ethylhydrocuprein discontinued on account of eye symptoms; eye symptoms disappeared
10 Hrs.	10	None	None	Spread to right upper	Recovery	
12 Hrs.	12	None	None	Spread to right lower	Death	Blood culture shortly before death yielded innumerable colonies
6.5 Hrs.	?	None	Empyema	Spread to left lower	Operation; death 60 days after admission	Blood culture became negative; intermission of 90¾ hours in ethylhydrocuprein treatment after 2.5 gm. had been given
Within 24 Hrs.	6	Temporary deafness	None	None	Recovery	
Within 24 Hrs.	6	Slight transient blurring of vision after temp. had become normal	None	None	Recovery	
Not studied	9	Temporary deafness	Delayed resoluton	None	Recovery	
Within 12 Hrs.	8	None	None	Spread to left lower	Death	
Within 12 Hrs.	?	Temporary blindness	None	None	Recovery	Ethylhydrocuprein discontinued on account of visual symptoms
Not studied	12	None	None	None	Recovery	Ethylhydrocuprein discontinued because it was thought that patient had had crisis
Within 12 Hrs.	8	None	None	None	Death	Strain obtained just after death was not "fast" to ethylhydrocuprein; pericardial fluid obtained post-mortem showed pneumococcicidal action for Type I pneumococcus

comparative impenetrability of a solid pneumonic exudate. Under such circumstances, as we have shown, pneumococci may become resistant or "fast" to the action of the drug.

As our experiments indicate, there seems to be some relationship between the number of pneumococci and the concentration of ethylhydrocuprein which is required to completely kill them. Thus, in test tube experiments it has been shown that whereas a given amount of the drug in solution is sufficient to destroy a given number of pneumococci per unit volume, the same amount of drug is not able to destroy 100 times this number in the same volume. Inasmuch as we cannot estimate in any human case the number of pneumococci which it is necessary to destroy, it is quite conceivable that much larger amounts of the drug than we have found it safe to administer may be necessary to produce the required concentration in the body fluids.

Finally, it is probable that in the concentration which may safely be attained in the blood stream of the patient (about 1 in 500,000), the drug does not penetrate the alveolar exudate to any marked degree and therefore cannot kill the pneumococci there present.

Our conception of the present status of ethylhydrocuprein therapy in lobar pneumonia is, then, that while much of the experimental evidence is favorable, the clinical results that have been obtained are scarcely sufficient to warrant the routine administration of a drug the use of which may result in damage to vision. Probably the drug would be efficient if it could be given in larger amounts. As a "lead" in chemotherapy the drug is of great value; synthetic study of the quinin alkaloids should be made for a compound possessing greater pneumococcidal power in the presence of body fluids, greater velocity of action on pneumococci and less toxicity. Such a compound should, in addition, possess the power of rapid and easy penetration into the alveolar exudate. With such a drug at our disposal we might expect in lobar pneumonia something approaching a *therapia sterilisans magna*.

CONCLUSIONS

1. Ethylhydrocuprein (optochin) base is absorbed with difficulty into the blood stream from the gastro-intestinal tract; the hydrochlorid of the drug is readily absorbed.

2. During treatment with ethylhydrocuprein pneumococci in the human body can gradually become "fast" or resistant to its action.

3. The pericardial fluid obtained post mortem from patients treated with ethylhydrocuprein hydrochlorid showed pneumococcidal power.

4. The serum of one patient who received a very large dose of ethylhydrocuprein hydrochlorid intramuscularly showed pneumococcidal power (Case 2947, Table 8).

5. Among seventy-five patients treated with ethylhydrocuprein there were nine who showed some degree of amblyopia (12 per cent.); this was mild in three cases and more severe in six. In all those who recovered from the pneumonia, the eye symptoms disappeared completely after the administration of the drug had been discontinued.

6. The systematic use of ethylhydrocuprein (optochin) in the treatment of seventy-five cases of acute lobar pneumonia due to pneumococci did not lead to any noteworthy therapeutic benefit. The failure of the ethylhydrocuprein treatment to influence favorably the course of the disease is probably due to the following:

(*a*). It is impossible to administer a sufficient amount of the drug to produce an effective concentration in the blood stream without at the same time exposing the patient to the danger of toxic action.

(*b*). The rate of the pneumococcidal action of ethylhydrocuprein is too slow in the concentrations which may be attained in the blood stream of the patient with any degree of safety; pneumococci, therefore, may gain access to the circulating blood at a greater rate than they are destroyed therein, even though the serum show pneumococcidal action.

(*c*). In the concentrations which are safely attained in the body fluids the drug probably penetrates but poorly into the alveolar exudate.

7. The routine use of ethylhydrocuprein in the treatment of acute lobar pneumonia cannot be recommended.

CIRCULATORY REACTIONS TO EXERCISE DURING CONVALESCENCE FROM INFECTIOUS DISEASE *

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The return of patients to normal after pneumonia, typhoid and typhus fevers and the other infectious diseases is a phenomenon with which we all are familiar clinically, yet convalescence up to the present has not been investigated in any exact or quantitative way. The length of time during which convalescent patients are confined to bed and the resumption of normal life are graduated very differently by different practitioners. In view of this great variation in procedure any accurate or quantitative method by which we could observe the stage of a patient's convalescence would be very desirable.

At present the need for such an accurate method of following a patient's convalescence is rendered acute by the military situation. We have in training a great number of young men, many of whom are being attacked by infectious diseases. In the treatment of these patients efficiency demands that their absence from military duties shall be sufficient for complete convalescence but shall not be prolonged unduly beyond the proper time. If the proper time for convalescence is to be determined, as it is at present, solely by the opinion of the attending physician, it is highly probable that some soldiers will be returned to active service too soon and some too late. In the former case we may do the patient serious injury; in the latter case we shall have wasted time, attention and hospital accommodation at a time when all of these are in great demand. With these considerations in mind we have conducted a series of experiments on patients recovering from acute infections.

We have confined our attention to the circulatory system because of the following considerations: Once the original infection has been overcome, the recovery of the patient means really the recovery of the patient's ability to do muscular work, and the recovery of the patient's ability to do muscular work is essentially a circulatory rather than a muscular phenomenon. The ordinary person in health overtaxes his circulation long before he exhausts his skeletal musculature. The convalescent from infectious disease is limited in his exercise not by what his muscles can do but what his heart can do. This is obvious when we consider that the serious pathologic effects of overexertion, both in health and in disease, are not muscular but circulatory. We cannot

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* From the cardiographic laboratory of the Mount Sinai Hospital.

easily overwork a skeletal muscle, because it has a very efficient safety device—refusal to respond. We can overwork the circulatory system, because refusal to respond adequately on its part does not result in immediate cessation of work. Therefore, it seemed logical to determine the circulatory reactions following muscular work at different periods during convalescence to see if we could discover any change in these reactions which might afford a criterion of the return to normal of the circulation.

The patients whose recovery we have followed have all been men between the ages of 21 and 45 years. There are ten cases in our series: seven pneumonias; one pleurisy; one typhoid fever; one typhus fever.

Our procedure has been as follows: The pulse rate was taken several times until it reached a constant figure. The systolic blood pressure was read by auscultation (using a mercury sphygmomanometer) until it reached its normal level. Then the patient performed a definite amount of work. The pulse rate was counted for 15 seconds immediately after the work and, at the end of 110 seconds, it was counted again for 20 seconds. From these two figures the rates immediately after exercise and at the end of 120 seconds were calculated. The systolic blood pressure was taken by the method described by Barringer[1] and also, in some cases, by the method of Cotton, Rapport and Lewis.[2] Our exercises have consisted in sitting up in bed and in flexing and swinging dumb-bells of various weights. We have calculated the work done in foot-pounds. The calculation of work done is fairly accurate and the error is constant for the same patient, so that slight inaccuracies will not vitiate our conclusions.

The method of Barringer[1] consists in taking the systolic pressure before exercise and then taking three readings after exercise—the first between 25 and 30 seconds; the second between 55 and 60 seconds; the third between 85 and 90 seconds—the endeavor being to make the readings as close to 30, 60 and 90 seconds as possible. The method described by Cotton, Rapport and Lewis[2] consists in taking the first reading as soon after exercise as possible and in taking numerous readings thereafter at very short intervals. These readings, when plotted, give us a curve which shows the variations in the systolic pressure after exercise. For reasons which we give later we have used Barringer's method in the majority of our experiments. Our technic has been standardized during the past four months by testing the circulatory reactions of a number of normal persons and of many patients suffering from cardiac insufficiency.

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1. Barringer, T. B., Jr.: Studies of the Heart's Functional Capacity, The Archives Int. Med., 1917, **20,** 829.

2. Cotton, T. F.; Rapport, D. L., and Lewis, T.: After Effects of Exercise on Pulse Rate and Systolic Blood Pressure in Cases of "Irritable Heart," Heart, 1917, **6,** 269.

Barringer believes that a "delayed rise" ("delayed summit," Cotton, Rapport and Lewis) indicates the overtaxing of the cardiac reserve power. A reading at sixty seconds after exercise which is 4 mm. or more higher than the reading at thirty seconds has been taken as indicative of a delayed summit. We have generally been able to produce a delayed summit much more pronounced than this minimum.

The following typical normal series of tests will illustrate the method of testing and recording. The subject was a normal man, 26 years old, weighing 160 pounds.

His systolic blood pressure at rest was..... 130
He swung two 10-pound dumb-bells 10 times
(Calculated work = 2,400 foot-pounds)
His systolic pressure after work was—at 30 seconds.... 150
at 60 seconds.... 140
at 90 seconds.... 130
In 5 minutes his systolic pressure at rest was constant at 120
He swung two 10-pound dumb-bells 25 times
(Calculated work = 6,000 foot-pounds)
His systolic pressure after work was—at 30 seconds.... 152
at 60 seconds.... 152
at 90 seconds.... 144
In 5 minutes his systolic pressure at rest was constant at 125
He swung two 10-pound dumb-bells 30 times
(Calculated work = 7,200 foot-pounds)
His systolic pressure after work was—at 30 seconds.... 150
at 60 seconds.... 164 (delayed summit)
at 90 seconds.... 156

In tabulated form the record reads as follows:

130	120	125	
2 × 10 S. 10 (2,400)	2 × 10 S. 25 (6,000)	2 × 10 S. 30 (7,200)	
30..... 150	30..... 152	30..... 150	(delayed summit)
60..... 140	60..... 152	60..... 164	
90..... 130	90..... 144	90..... 156	

Graphically the record would appear in Chart 1.

We can express the fact that the subject showed a delayed summit after doing 7,200 foot-pounds of work and did not show a delayed summit after 6,000 foot-pounds of work as in Chart 2.

We have omitted any mention of the time in which the work is done. Patients soon acquire a regular rhythm in working, the time of each swing being the same (2 to 3 seconds). Thus, the time factor becomes a constant and can be left out of consideration.

The accompanying series of charts (Charts 3, 4, 5 and 6) shows the change in reactions of the blood pressure to muscular work during convalescence.

It will be observed that all the convalescent patients show the same phenomenon—a progressive increase in the amount of work that can be done without causing a delayed summit of blood pressure. This increase in all cases was synchronous with subjective symptoms of

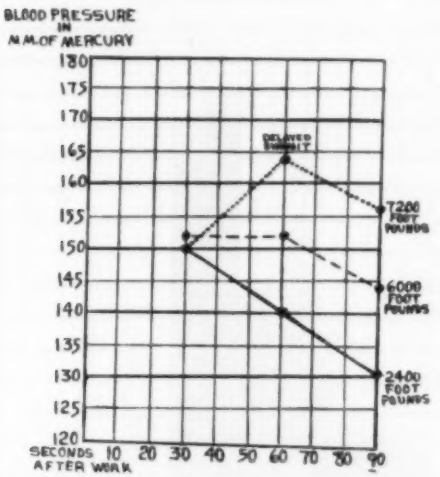

Chart 1.—This chart shows a typical series of blood pressure readings after increasing amounts of work. The subject was a normal man 26 years old. Note that after a small amount of work the pressure falls rapidly; after a greater amount of work the return to normal is not so rapid; after a still greater amount of work the blood pressure continues to increase for some time—"delayed summit." The blood pressure before exercise was about 125.

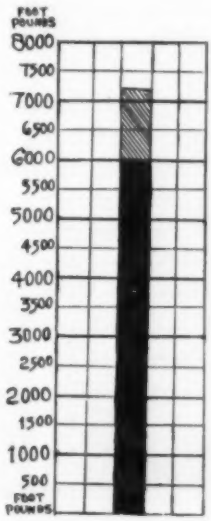

Chart 2.—This chart indicates that with 6,000 foot-pounds of work or less there is no delayed summit; with 7,200 foot-pounds of work or more there is a delayed summit; between 6,000 and 7,200 foot-pounds the circulatory reaction to work changes.

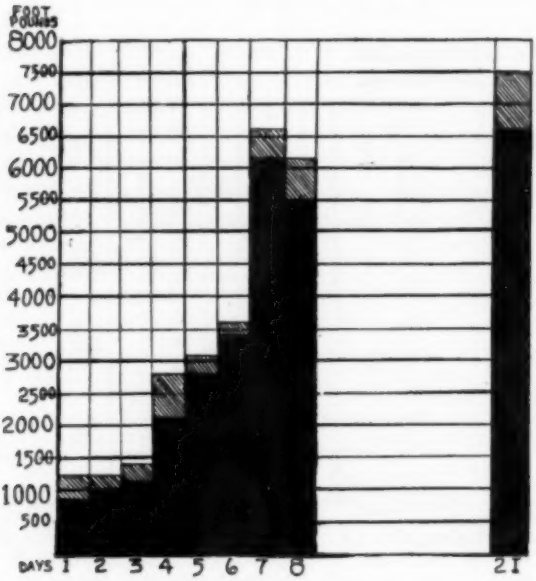

Chart 3

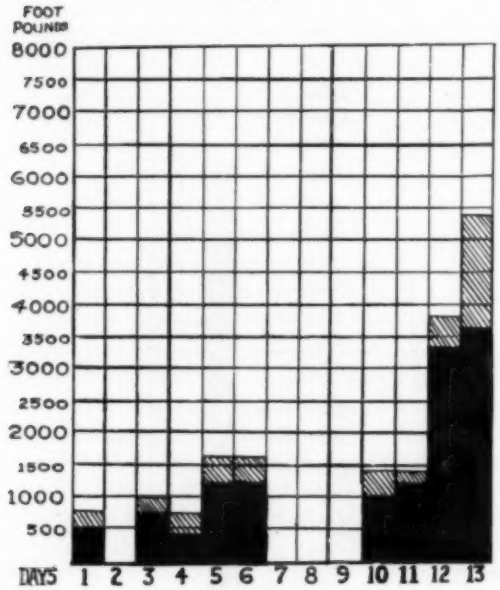

Chart 4

Charts 3 and 4.—These charts show the change in the circulatory reaction to exercise which takes place during convalescence. Chart 3 is from the patient I. M. in Table 1; Chart 4 is from the patient C. B. in Table 1.

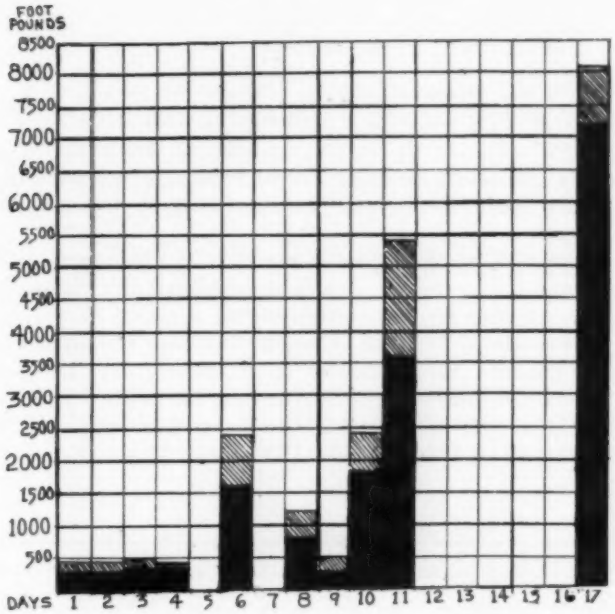

Chart 5

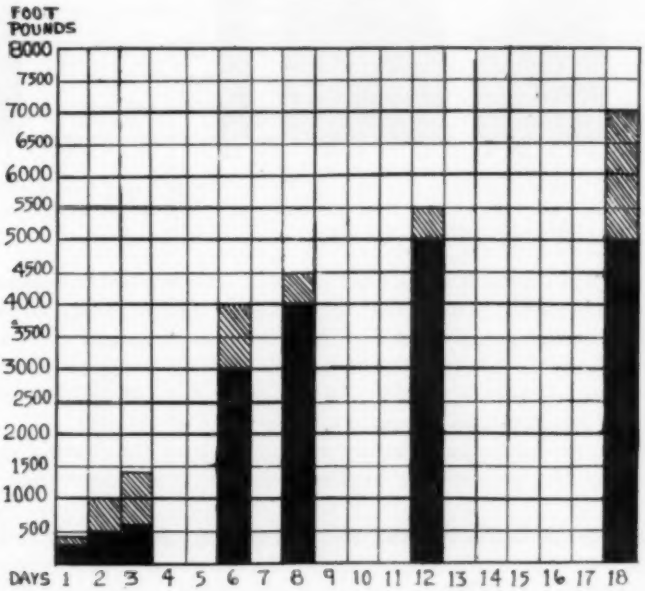

Chart 6

Charts 5 and 6.—These are like charts 3 and 4. They show the same progressive change in circulatory reactions. Chart 5 is from the patient R. F. in Table 1; Chart 6 is from the patient M. O. in Table 1.

improvement and increased activity. Patients R. F. and C. B., who show a late development of this phenomenon, were subjectively weak and improved very slowly before the time at which the objective improvement in the circulatory reactions began. Synchronously with the objective improvement there was marked subjective and clinical improvement.

In the cases I. M., C. B., R. F. and M. O., which were followed carefully with daily readings, it will be noted that this change in the circulatory reactions is most marked during a period of a very few days. In the case of I. M. the change in four days was from 1,500 to 6,500 foot-pounds. In the case of R. F., in two days the point at which the delayed summit appeared rose from 500 to 5,000 foot-pounds. C. B. rose from 1,500 to about 5,000 foot-pounds in two days. M. C. changed from about 1,000 to above 3,000 in two days.

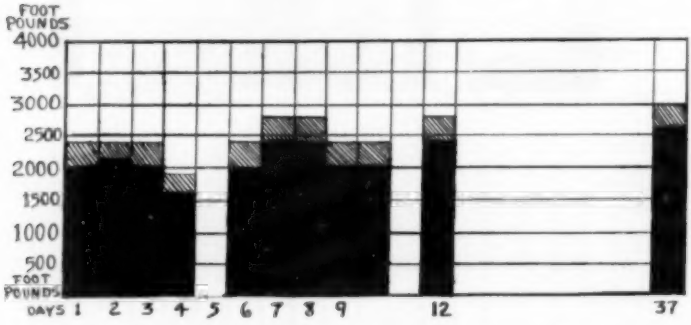

Chart 7.—This chart shows the circulatory reactions to exercise in a normal woman taken day after day. Note that the daily variation is comparatively slight. Compare with Charts 3, 4, 5 and 6.

That this change is not a mere result of the exercises to which the patient is subjected in the process of trying out his circulatory reactions is shown by the control Chart 7. The control was a normal woman not used to exercise. Observe that there is no marked change produced by the amount of exercise necessary to try out her circulatory reactions.

Table 1 summarizes our results in this series of patients.

When the method of frequent readings is used we obtain a result something like that shown in Chart 8, which is taken from the case R. F.

Both the slow readings and the rapid readings are given, and it will be observed that they both give exactly the same conclusions as regards the circulatory reactions. The readings taken at thirty, sixty and ninety seconds with the rapid method compare well with the same readings taken with the infrequent method. We have observed this

TABLE 1.—Change in Reaction of the Systolic Pressure to Exercise During Convalescence *

Patient	Age	Disease	Recovery	Circulatory Reactions to Exercise Days									
I. M.	22	Lobar pneumonia	Crisis —9 days	1 1,200 900	2 1,200 1,000	3 1,400 1,120	4 2,800 2,100	5 3,080 2,800	6 3,600 3,420	7 6,600 6,160	8 6,160 5,500		21 7,500 6,600
C. B.	40	Lobar pneumonia	Crisis —6 days	1 750 500	2 1,000 750	4 750 400	5 1,000 1,200	6 1,000 1,200	10 1,400 1,000	11 1,400 1,200	12 3,800 3,300	13 5,400 3,600	
R. F.	41	Lobar pneumonia	Lysis —6 to —3 days	1 450 300	2 450 300	3 500 460	4 450 360	6 2,400 1,600	8 1,200 800	9 500 300	10 2,400 1,800	11 5,400 3,600	17 8,100 7,200
M. O.	22	Lobar pneumonia	Crisis —3 days	1 450 300	2 1,000 500	3 1,400 600	5 4,000 3,000	8 4,500 4,000	12 5,500 5,000				18 7,000 5,000
B. M.	44	Typhus	Crisis —9 days	1 1,300 1,100					6 2,500 2,100				
B. B.	27	Lobar pneumonia	Crisis —12 days	1 2,300 1,800						7 3,500 3,000			
C. G.	22	Fibrinous pleurisy		1 200 0			4 2,200	5 3,500					
J. O.	21	Typhoid	Lysis —1 to +2 days	1 600	2 1,100 600			5 1,100				9 2,600 1,800	20 5,300
W. M.	..	Lobar pneumonia	Crisis —3 days	1 400 200						7 1,400 800		9 1,600 1,200	
H. R.	21	Lobar pneumonia	Crisis —15 days	1 1,900 1,500		3 4,000 3,200	4 5,000 4,000						

* The lower numeral of each pair gives the greatest amount of work, calculated in foot-pounds, which was not followed by a delayed summit. The upper numeral gives the smallest amount of work which was followed by a delayed summit. For example: Patient I. M. was tested for the first time nine days after his crisis and showed a delayed summit with 1,200 or more foot-pounds of work, while with 900 foot-pounds or less he showed no delayed summit. Twenty-one days later he showed a delayed summit with 7,500 foot-pounds of work or more and no delayed summit with 6,600 foot-pounds or less. Note: The patients, I. M., C. B., R. F., and M. O., have their reactions represented graphically in Charts 3, 4, 5 and 6, respectively.

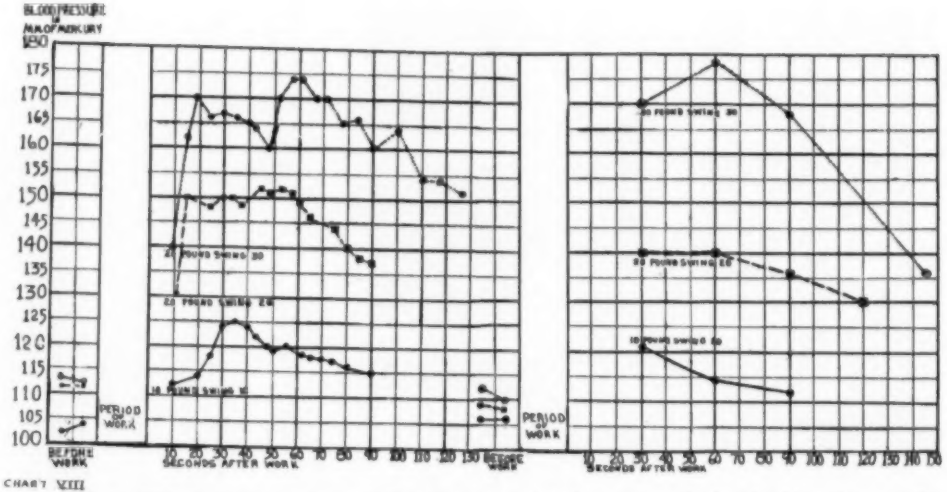

CHART VIII

Chart 8.—This is a comparison of the frequent and infrequent methods of taking the systolic pressure after exercise. Note that the readings taken at thirty, sixty and ninety seconds by both methods give curves which correspond very closely. The work was all done on the same day and the patient rested from five to ten minutes after each exercise.

same correspondence whenever we have compared the two methods. The infrequent or slow method, while it does not give us such detailed information about the exact shape of the curve, does give us the information which we have found to be of the most value; that is, whether or not there is a delayed summit. It is a method which is much easier to use, since it requires only one examiner, while, with the frequent reading method, two persons are required, one to read and one to record. For practical work the Barringer method has the advantage of simplicity combined with adequacy.

EFFECT OF EXERCISE ON THE PULSE RATE

Table 2 shows the pulse reactions to exercise of nine patients who were recovering from infectious diseases. The presence or absence of a delayed summit of systolic pressure after exercise is also recorded for purposes of comparison. It will be observed that the table shows thirty-two delayed summits, twelve of which show a delay in the return of the pulse to normal, while in the other twenty there is no such delay shown.

In general, the rate increases with increased amounts of exercise and takes an increasingly longer time to return to normal with increased amounts of work, but this general rule has many exceptions, especially in patients who show a marked slowing after exercise, probably due to nervous influence on the heart rate.

We have been able to make no deductions of value from the effects of work on the pulse rate or from the time required for the rate to return to normal after work in this small series of patients.

The agreement of our objective circulatory findings with subjective and clinical changes in the patients whom we have examined has been very striking. Our observations on different convalescent patients have shown a marked similarity and the results of these experiments afford evidence of considerable importance in support of the contention advanced by Barringer[1] that a delayed summit indicates an overtaxing of the cardiac reserve power.

Any method which will permit of fairly accurate objective measurement of the stage of convalescence has abundant possibilities of development and of use, both in the immediate present and in the future. It is most important at present that the convalescence of our soldiers be expedited as much as is consistent with good therapeutics. For the maximum of efficiency in this direction some standard objective method of testing the soldier's ability to resume active service is of great importance. Such a method these experiments seem to indicate.

So far the method of testing the circulatory reactions to exercise has been applied only to patients convalescing from acute infections. There is considerable probability of our getting useful information from the application of this method of study to patients who are recovering from the more chronic affections.

TABLE 2.—Effect of Work on the Pulse Rate of Patients During Convalescence *

Patient	Work	Pulse Rate		Return to Normal, Seconds	Delayed Summit	Delay, Seconds
		Before	Immediately After			
M. O.	Times					
	Sat up 5	68	86	180	No	
	Sat up 10	68	92	180	No	
	Sat up 15	80	88	120	No	
	7 lb. swing 5	96	108	120	No	
	7 lb. swing 10	88	108	120	No	
	10 lb. swing 10	90	114	120	Yes	58
	7 lb. swing 5	92	96	120	No	
	7 lb. swing 10	88	104	120	No	
	10 lb. swing 10	84	108	120	No	
	10 lb. swing 15	84	112	120	No	
	20 lb. swing 15	84	124	180	No	
	10 lb. swing 5	96	108	180	No	
	10 lb. swing 10	92	108	180	No	
	10 lb. swing 10	92	112	120	No	
	10 lb. swing 20	98	124	180	No	
	20 lb. swing 20	96	144	180	Yes	60
	20 lb. swing 10	104	128	120	No	
	20 lb. swing 15	104	140	180	No	
	20 lb. swing 25	104	158	180	Yes	60
	20 lb. swing 35	104	156	240	Yes	60
W. M.	Sat up 5	58	60	60	No	
	Sat up 10	60	72	120	Yes	60
	10 lb. swing 25	80	116	90	No	
	20 lb. swing 20	84	132	120	Yes	60
	20 lb. swing 15	90	112	118	No	
	20 lb. swing 20	96	124	120	Yes	60
	20 lb. swing 25	96	128	120	Yes	60
C. B.	20 lb. flex 10	68	84	120	Yes	60
	20 lb. flex 20	68	88	120	Yes	60
	10 lb. flex 15	68	84	60	No	
	10 lb. flex 20	72	80	120	No	
	10 lb. flex 30	74	92	120	No	
	10 lb. swing 10	72	100	120	No	
	10 lb. swing 30	84	108	120	No	
	20 lb. swing 20	72	132	240	No	
	20 lb. swing 30	76	144	150	Yes	60
	20 lb. flex 15	50	76	120	No	
	20 lb. flex 20	54	72	90	Yes	60
	20 lb. flex 30	54	76	120	Yes	60
R. F.	Sat up 10	76	82	120	No	
	Sat up 12	72	88	120	No	
	Sat up 15	72	88	120	Yes	61
	10 lb. swing 10	64	88	120	No	
	20 lb. swing 20	68	112	120	No	
	20 lb. swing 30	68	128	240	Yes	35
	20 lb. swing 30	88	120	150	No	
	20 lb. swing 35	84	136	240	No	
	20 lb. swing 40	84	140	300	No	
	20 lb. swing 45	84	148	360	Yes	60

* The exercises in each group were given on the same day and five to ten minutes intervened between individual exercises. The figure 120 in the column headed "Return to Normal" means that the rate became normal in two minutes or less.

TABLE 2.—Effect of Work on the Pulse Rate of Patients During Convalescence *—(*Continued*)

Patient	Work	Pulse Rate		Return to Normal, Seconds	Delayed Summit	Delay, Seconds
		Before	Immediately After			
	Times					
E. W.	Sat up 10	52	72	180	Yes	90
	Sat up 15	52	72	180	Yes	90
	Sat up 20	48	72	120	Yes	150
	Sat up 10	40	48	120	Yes	90
	Sat up 15	40	52	120	Yes	90
J. O.	7 lb. flex 21	112	128	120	Yes	90
	7 lb. flex 30	112	136	250	Yes	210
	7 lb. swing 5	96	112	120	Yes	60
	7 lb. swing 10	104	124	120	Yes	120
	10 lb. swing 5	108	120	180	No	
	10 lb. swing 5	96	112	120	No	
	10 lb. swing 10	92	108	180	No	
	10 lb. swing 15	100	120	120	No	
	10 lb. swing 20	90	92	180	Yes	58
	10 lb. swing 10	112	120	120	No	
	10 lb. swing 15	112	124	120	No	
	10 lb. swing 20	108	136	180	No	
	20 lb. swing 20	112	156	300	No	
	20 lb. swing 25	120	160	180	No	
	30 lb. swing 30	116	168	300	No	
I. F.	10 lb. swing 5	72	88	120	No	
	10 lb. swing 10	72	104	180	No	
	10 lb. swing 15	76	104	180	No	
	10 lb. swing 20	88	88	300	Yes	120
J. E.	10 lb. swing 10	76	88	120	No	
	20 lb. swing 10	72	92	180	No	
	20 lb. swing 15	80	108	120	Yes	60
	20 lb. swing 15	72	92	120	No	
	20 lb. swing 20	76	100	120	Yes	60
C. G.	10 lb. flex 10	112	116	120	Yes	60
	10 lb. flex 15	108	112	180	Yes	61
	10 lb. flex 10	96	120	180	No	
	10 lb. flex 15	104	104	180	No	
	10 lb. flex 20	100	108	120	No	
	10 lb. flex 25	104	104	180	No	
	15 lb. flex 20	100	136	180	No	
	10 lb. swing 10	112	116	120	No	
	10 lb. swing 15	112	120	120	No	
	10 lb. swing 10	104	116	120	No	
	10 lb. swing 20	104	116	120	Yes	60

SUMMARY

1. The circulatory reactions of ten patients convalescing from acute infectious disease have been studied objectively.

2. The pulse reactions have not given us any information of value.

3. The blood pressure reactions have shown a progressive increase in the amount of work necessary to produce a "delayed summit." This increase has been shown in all cases, has been especially marked in a short period of a very few days, and has been synchronous with clinical and subjective improvement.

CONCLUSION

The reaction of the systolic blood pressure to exercise in a convalescent patient affords valuable objective evidence of the stage of the patient's convalescence.

I wish to express my indebtedness to Dr. Alfred Meyer, Dr. Emanuel Libman, and Dr. Morris Manges for permission to follow the convalescence of patients on their wards.

THE OCCURRENCE OF MITOCHONDRIA IN THE RED BLOOD CORPUSCLES DURING EXPERIMENTAL ANEMIAS *

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INTRODUCTION

Mitochondria are small bodies of a lipoid nature which occur in the cell protoplasm and which may be stained in a specific manner by a variety of methods.[1] They possess no fixed morphologic characteristics, but appear either as granules of varying size or as tiny rods. They are said by some to be present in all nucleated cells.

Mitochondria are not demonstrable in the circulating nonnucleated erythrocytes of healthy adult mammals. Shipley[1] and others have shown, however, that they are regularly present in nucleated red cells. In such cells they have been observed in the circulating blood of lower vertebrates, in the circulating blood of mammalian embryos and in the bone marrow of adult mammals. Of more immediate interest is the fact that mitochondria have also been demonstrated in certain nonnucleated erythrocytes under conditions in which one would expect to find youthful cells of this type. For example, nonnucleated red cells that contained mitochondria have been demonstrated in the bone marrow and circulating blood of mammalian embryos, in the bone marrow of normal mammalian adults and in the circulating blood of man during the course of some diseases in which the blood-forming organs are supposedly stimulated to increased activity. Their presence in nonnucleated erythrocytes, therefore, appears to indicate that such erythrocytes are relatively immature.

The present study was undertaken in order to test this hypothesis further. Anemias were produced experimentally in rabbits by bleeding or by the injection of phenylhydrazin hydrochlorid. Such measures lead to an increased activity on the part of the erythroblastic tissues and to the escape of more or less immature red cells into the circulating blood. One might anticipate, therefore, that in such anemic animals mitochondriated red cells would appear in the circulating blood owing to the escape of immature forms from the bone marrow.

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* From the Division of Medicine, Stanford Medical School.

1. Cowdry, E. V.: The Vital Staining of Mitochondria with Janus Green and Diethylsafranin in Human Blood Cells, Internat. Monatschr. f. Anat. u. Physiol., 1914, **31,** 267; the General Functional Significance of Mitochondria, Am. Jour. Anat., 1916, **19,** 423. Shipley, P. G.: The Mitochondrial Substance in the Erythrocytes of the Embryo Pig. Fol. Haem., 1916, **20,** 61.

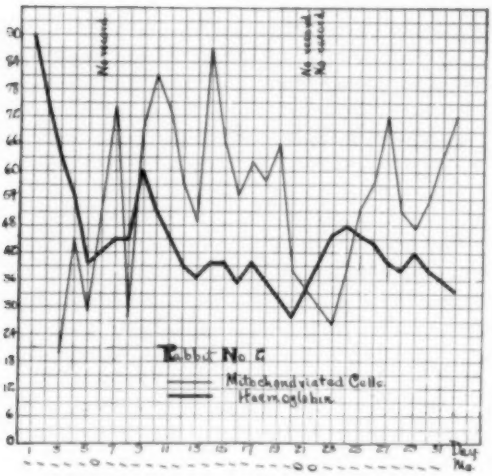

Chart 1.—Rabbit 2. Injections as noted.

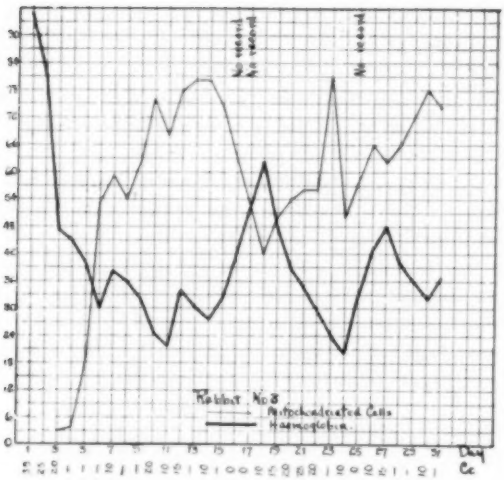

Chart 2.—Rabbit 3.—Injections as noted.

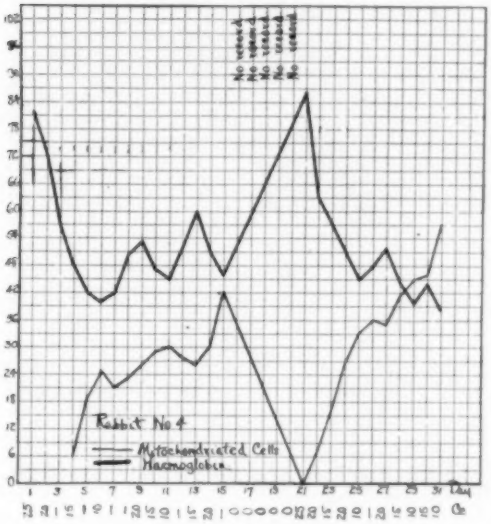

Chart 3.—Rabbit 4. Bleeding as noted (—is ditto).

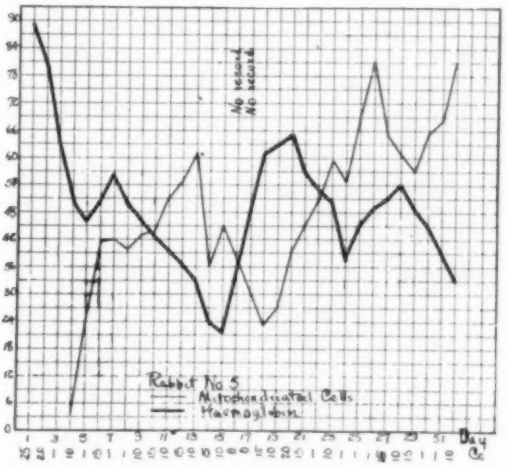

Chart 4.—Rabbit 5. Bleeding as noted (— is ditto).

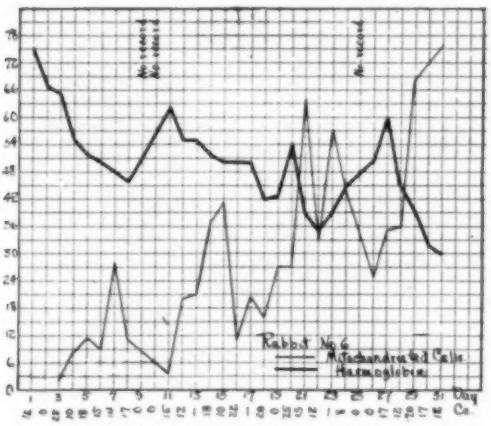

Chart 5.—Rabbit 6. Bleeding as noted (— is ditto).

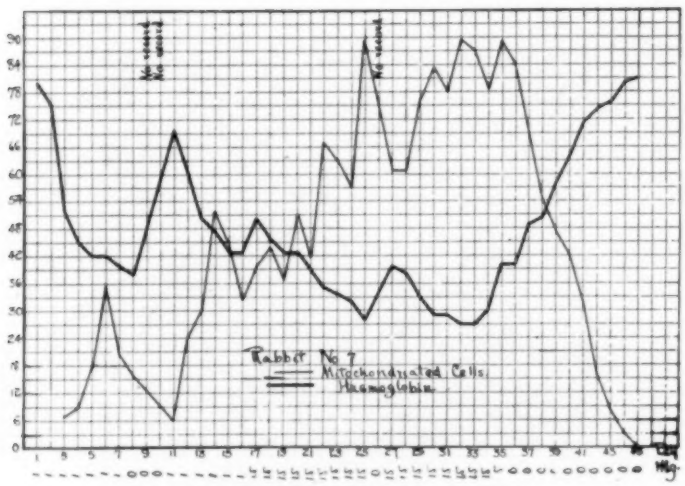

Chart 6.—Rabbit 7. Injections as noted.

Technic.—As has been said, the rabbits used in this study were made anemic either by bleeding or by the injection of phenylhydrazin hydrochlorid. When the former method was used, from 10 to 30 c.c. of blood were withdrawn daily; when the latter was used, from 1 to 1.5 mg. of the the poison were injected daily. In both cases, however, partial or complete recoveries were at times permitted by omitting the bleedings or the injections for one or more days during the experiments.

The hemoglobin percentage was determined almost daily by the Sahli hemoglobinometer. Smears stained by Wright's method were also examined in some of the experiments in order to compare the number of mitochondriated cells with such alterations in the red corpuscles as were demonstrable by this method.

TABLE 1.—DATA OF EXPERIMENTS, RABBIT 2, FEMALE, WEIGHT 2.2 KG.

Day	Hemoglobin, Per Cent.	Mitochondriated Cells	Pheynlhydrazin, Mg.	Stained Smear
1	90	0	1	No change
2	76	0	1	No change
3	65	20	1	Many kernschatten; moderate amount of polychromatophilia
4	55	46	1	Slight anisocytosis and polychromatophilia; a rare normoblast
5	40	29	1	A few polychromes; very slight poikilocytosis; a rare normoblast
6	No record	..	..	No record
7	45	74	1	Slight polychromatophilia and basophilic degeneration; marked anisocytosis
8	45	28	1	Moderate amount of poikilocytosis, anisocytosis
9	60	70	1	Slight polychromatophilia
10	50	81	1	Cells large; no normoblasts
11	45	73	1	A few polychromes and basophils; no normoblasts
12	40	57	1	No other changes
13	37	49	1	Some polychromatophilia
14	40	87	1	No change
15	40	66	1	A few polychromes
16	35	55	1	Small amount of anisocytosis
17	40	62	1	A few polychromes
18	36	58	1	A few large cells
19	32	66	1	No normoblasts seen
20	28	38	1	Slight poikilocytosis
21	No record	..	..	No record
22	No record	..	..	No record
23	46	26	1	Polychromatophilia; a rare normoblast
24	48	40	1	Many large cells; some poikylocytosis
25	46	52	1	Some polychromatophilia
26	44	58	1	Some anisocytosis
27	40	72	1	Some basophilic cells; a few polychromes
28	38	51	1	Slight anisocytosis; many large cells
29	42	47	1	No other changes
30	38	53	1	No other changes
31	36	63	1	Slight polychromatophilia
32	34	72	1	No other changes

The following technic for staining mitochondria was used. A stock solution of Janus Green B (Farbwerke-Hoechst Co., New York) was made in water in a concentration of 1 to 2,000. Before use this was mixed with a stock solution of sodium chlorid (1.08 per cent.) in the proportion of one part of stain to two parts of the salt solution. A drop each of blood and of the staining mixture were placed on a slide and immediately covered with a cover slip. In such preparations mitochondria appeared as dancing granules or rods of a bright green color.

To determine the proportion of mitochondria red blood cells preparations were placed under the oil immersion lens of a Leitz microscope (ocular 2). A 60 watt tungsten light with frosted globe furnished the illumination. A number of evenly distributed fields in different parts of each preparation were selected, the total cells counted and the mitochondriated cells separately enumerated. From these figures the percentage of the latter was computed. In each set of preparations from 500 to 1,000 cells were counted.

TABLE 2.—Data of Experiments, Rabbit 3, Female, Weight 1.5 Kg.

Day	Hemoglobin, Per Cent.	Mitochondriated Cells	Blood Taken, C.c.	Stained Smear
1	95	0	21	No changes
2	80	0	25	Many kernschatten; a few polychromes
3	47	3	20	No visible changes
4	45	4	20	Marked anisocytosis and polychromatophilia; many large cells
5	40	18	20	Anisocytosis and polychromatophilia not so marked
6	30	53	20	No changes
7	38	59	10	Slight poikilocytosis; no other changes
8	36	54	15	Slight amount of basophilic stippling
9	32	62	15	Many large cells; many polychromes and basophils
10	25	76	20	Smear about the same
11	22	68	10	Some anisocytosis
12	34	78	15	A rare normoblast; some polychromatophilia
13	30	80	15	Slight poikilocytosis
14	28	80	10	A rare normoblast
15	32	74	10	Some polychromatophilia and anisocytosis
16	No record	..	..	No record
17	No record	..	..	No record
18	62	42	10	Slight poikilocytosis
19	47	50	15	Slight anisocytosis and polychromatophilia
20	39	54	20	Slight polychromatophilia
21	35	58	25	Slight basophilic degeneration
22	30	58	20	Much polychromatophilia
23	24	81	20	Some anisocytosis and polychromatophilia
24	20	50	10	A very rare normoblast; slight amount of polychromatophilia
25	No record	..	..	No record
26	43	66	10	Slight poikilocytosis
27	48	62	15	Slight anisocytosis
28	40	66	15	No changes
29	36	72	15	Slight polychromatophilia
30	32	78	10	No visible changes
31	40	74	10	Marked anisocytosis

TABLE 3.—Data of Experiments, Rabbit 4, Female, Weight 2.4 Kg.

Day	Hemoglobin, Per Cent.	Mitochondriated Cells, Per Cent.	Blood Taken, C.c.
1	82	0	25
2	72	0	20
3	62	0	20
4	48	5	15
5	42	18	15
6	40	25	10
7	42	21	10
8	50	23	10
9	53	26	20
10	47	29	15
11	45	30	10
12	52	28	10
13	58	26	15
14	52	30	20
15	46	42	20
16	No record	..	..
17	No record	..	..
18	No record	..	..
19	No record	..	..
20	No record	..	..
21	88	0	25
22	64	7	20
23	56	16	15
24	53	23	20
25	46	33	10
26	48	36	10
27	52	35	20
28	44	41	15
29	40	45	10
30	44	46	15
31	38	57	10

TABLE 4.—Data of Experiments, Rabbit 5, Female, Weight 2.1 Kg.

Day	Hemoglobin, Per Cent.	Mitochondriated Cells, Per Cent.	Blood Taken, C.c.
1	89	0	25
2	79	0	20
3	62	0	20
4	50	4	10
5	46	23	10
6	50	41	15
7	56	42	15
8	50	40	15
9	46	43	10
10	43	45	15
11	40	51	10
12	37	55	15
13	33	61	10
14	25	36	15
15	22	45	10
16	No record	..	..
17	No record	..	..
18	61	23	15
19	63	27	10
20	65	39	20
21	57	45	15
22	53	50	15
23	50	59	10
24	38	55	10
25	45	69	10
26	49	81	10
27	51	65	15
28	54	59	10
29	49	55	15
30	45	65	15
31	39	68	15
32	33	81	10

TABLE 5.—Data of Experiments, Rabbit 6, Female, Weight 2.5 Kg.

Day	Hemoglobin, Per Cent.	Mitochondriated Cells	Blood Taken, C.c.	Stained Smear
1	75	0	12.5	No changes noted
2	67	0	0	No changes
3	65	2	25	No changes noted
4	55	8	10	Many kernschatten; slight polychromatophilia
5	52	11	18	Marked anisocytosis
6	50	9	15	Marked anisocytosis
7	48	28	14	No change
8	46	11	17	No change
9	No record	..	..	No record
10	No record	..	..	No record
11	62	4	15	Some poikilocytosis and polychromatophilia
12	55	20	12.5	Many kernschatten
13	55	21	12.5	Some poikilocytosis and anisocytosis
14	52	37	13	Marked polychromatophilia; some poikilocytosis
15	50	41	10	Slight polychromatophilia; 8 normoblasts seen
16	50	11	22	Some anisocytosis and polychromatophilia
17	50	20	22	
18	42	16	20	
19	43	27	0	Many kernschatten; some anisocytosis
20	54	27	25	
21	39	64	15	Marked poikilocytosis; a few polychromes
22	35	33	12.5	
23	39	57	12.5	Same except for decrease in poikilocytosis
24	45	44	8	
25	No record	..	..	Marked polychromatophilia; some anisocytosis
26	50	25	0	
27	60	35	17	Slight polychromatophilia
28	45	36	12	
29	39	68	20	Some anisocytosis
30	32	72	17	
31	30	76	12	Slight polychromatophilia

TABLE 6.—Data of Experiments, Rabbit 7, Female, Weight 1.8 Kg.

Day	Hemoglobin, Per Cent.	Mitochondriated Cells	Pheynlhydrazin, Mg.	Stained Smear
1	80	0	1	No changes noted
2	75	0	1	No changes noted
3	52	7	1	No change
4	45	9	1	Slight anisocytosis and polychromatophilia
5	42	17	1	Some anisocytosis
6	42	36	1	Some polychromatophilia
7	40	20	1	Slight poikilocytosis
8	38	16	0	Some poikilocytosis; a few polychromes
9	No record	..	..	No record
10	No record	..	..	No record
11	70	6	1	Marked anisocytosis and poikilocytosis
12	60	24	1	Some polychromatophilia
13	50	30	1	Marked polychromatophilia
14	47	52	1	Slight polychromatophilia
15	43	44	1	Slight polychromatophilia
16	43	32	1	
17	50	40	1.5	Slight anisocytosis
18	46	44	1.5	
19	43	37	1.5	Slight poikilocytosis and polychromatophilia
20	43	51	1.5	
21	39	41	1.5	Slight polychromatophilia
22	35	67	1.5	
23	34	63	1.5	Marked number of polychromes
24	32	57	1.5	
25	28	89	1.5	Some polychromatophilia
26	No record	..	..	
27	40	61	1.5	Slight anisocytosis
28	38	60	1.5	
29	33	76	1.5	A few polychromes
30	29	83	1.5	
31	29	78	1.5	Slight anisocytosis and poikilocytosis
32	27	89	1.5	
33	37	89	1.5	
34	30	81	1.5	
35	40	91	1.5	
36	40	86	0	
37	49	71	0	
38	50	54	0	
39	58	47	0	
40	64	42	0	
41	73	24	0	
42	76	16	0	
43	78	8	0	
44	82	3	0	
45	83	0	0	

TABLE 7.—Data of Experiments, Rabbit 8, Female, Weight 1.9 Kg.

Day	Hemoglobin, Per Cent.	Mitochondriated Cells, Per Cent.	Phenylhydrazin, C.c.
1	78	0	1.0
2	69	0	1.0
3	51	11	1.0
4	48	23	1.0
5	48	28	1.0
6	44	31	1.0
7	40	32	1.0
8	39	34	1.0
9	45	35	1.5
10	41	37	1.5
11	36	41	1.5
12	31	23	1.5
13	27	49	1.0
14	29	43	1.0
15	31	39	1.0
16	35	59	0
17	43	51	0
18	51	43	0
19	83	1	1.5
20	69	15	1.5
21	51	19	1.5
22	37	23	1.5
23	35	31	1.5
24	37	41	1.5
25	37	45	1.5
26	33	53	1.5
27	35	57	1.5
28	37	59	1.5
29	40	64	1.5
30	41	70	1.5
31	37	81	1.5

DISCUSSION

The details of the separate experiments can best be studied by referring to the accompanying tables and figures. It will be noted that in none of the animals were mitochondriated red cells seen previous to the production of the anemia and that in each animal these cells occurred after its production, rising in several instances to a maximum of over 80 per cent. of the total number of erythrocytes. By reference to the figures it is also evident that in a general way the percentage of hemoglobin and the proportion of mitochondriated red cells varied in

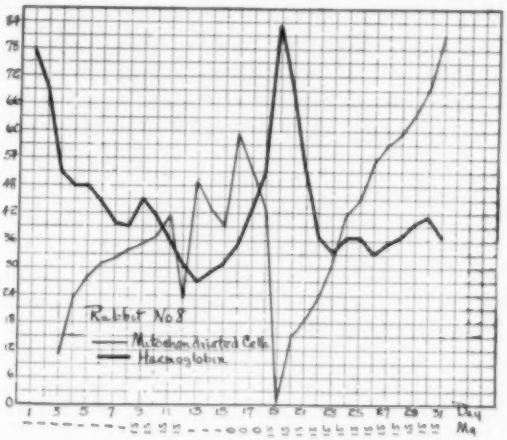

Chart 7.—Rabbit 8.—Injections as noted.

the inverse direction. In one instance (Rabbit 7) in which the hemoglobin was permitted to return to the normal, the mitochondriated cells gradually disappeared.

TABLE 8.—AVERAGE PERCENTAGE OF MITOCHONDRIATED CELLS AT DIFFERENT LEVELS OF HEMOGLOBIN

Number of Observations	Hemoglobin, Per Cent.	Average Per Cent. of Mitochondriated Cells	Per Cent. Range of Variation
12	60-65	27	35
9	55-59	30	19
29	50-54	33	38
33	45-49	40	38
37	40-44	48	37
32	35-39	52	33
19	30-34	65	29
13	25-29	69	26

The inverse relation between the proportion of mitochondriated cells and the percentage of hemoglobin could also be demonstrated in

another way. In Table 8 the average percentage of mitochondriated cells at different levels of hemoglobin is given. These averages were compiled from all the counts made, irrespective of whether the hemoglobin was decreased by bleeding or by the injection of phenylhydrazin.

No difference was noted in the relation between the percentage of mitochondriated cells and the hemoglobin concentration in the two different forms of anemia. The mitochondria in the phenylhydrazin anemia, however, were more deeply stained and larger.

Special attention was paid in certain experiments to the appearance of nucleated red cells and of polychromatophilic cells in smears stained by Wright's method. The occurrence of these cells was subject to considerable variation and no constant relation could be established between the finding of nucleated red cells, the degree of polychromatophilia and the percentage of mitochondria-containing cells.

CONCLUSIONS

If the presence of mitochondria in red blood cells is an indication that they are newly formed cells, one would expect to find an increase in their number whenever there is an increased rate of red cell formation. The need for increased formation may be produced experimentally by the removal of blood or by the injection of phenylhydrazin hydrochlorid.

The present study showed that these procedures were, in fact, accompanied by an increase in the number of mitochondria-containing red cells, and further that the degree of this increase was in the individual case roughly proportional, and on the average exactly proportional, to the need for red cells as measured by the degree of anemia produced.

These experiments, therefore, support the suggestion that the number of red cells containing mitochondria may prove to be a useful indication of the rate of red blood cell formation in clinical and experimental conditions.

The author wishes to acknowledge his indebtedness to Dr. Thomas Addis for suggestions and aid during the course of this investigation.